Nova Scotian Autumn Cod Spawning

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ABSTRACT

Investigation by means of commercial and experimental fishing with baited gear, and gill nets, shows that sexually mature cod enter Halifax and St. Margaret bays in early October at a time when the resident cod have moved out to the mouths of these bays.

Thereafter, until early December, these fish are taken in spawning condition on shoals at the heads of these bays.

Tows with plankton nets take both cod eggs and larvae in and around these bays until late December, but none thereafter until about April.

These autumn-spawning cod differ from the regular summer cod of these regions by having a much lower average vertebral count and by spawning in comparatively warm, shoal water at the heads of the bays instead of outside the bays in much cooler water in the late winter.

INTRODUCTION

The spawning of cod (*Gadus callarias* Linn.) in the autumn, as distinct from the more usual spawning in winter and spring, does not appear to have been especially considered by previous authors. This spawning in Nova Scotia takes place in certain inlets (principally Halifax harbour and St. Margaret bay) only.

During the course of the investigation, which has been carried on since 1933, Mr. C. K. Darrach of the Atlantic Fisheries Experimental Station and Capt. A. E. Calder of the M. V. "*Zoarces*", Atlantic Biological Station, have helped greatly by making numerous collections. Their assistance is gratefully acknowledged. The help of Dr. J. L. Hart and co-workers who reviewed the manuscript is acknowledged.

During the routine collection and examination of cod from Halifax harbour in 1933, it was found that the autumn samples differed greatly from those of the late summer in sexual development and average vertebral number. Sexually, it was observed that early in the autumn the degree of maturity of the cod taken changed suddenly from about half mature to almost "running," and coincident with this change a great drop was seen to occur in the average vertebral count.

SEASONS FOR COD SPAWNING

A survey of the literature shows the north Atlantic cod to be principally a winter and spring spawner, although spawning is protracted in many places.

Spawning thus occurs when the water temperature is at or rising from the yearly minimum. It takes place at a depth of thirty to one hundred metres, both inshore and offshore.

The period from February to May includes most of the spawning (from west to east in the north Atlantic: Georges bank, Bigelow and Welsh 1925; bay of Fundy, McKenzie 1934; off southwest Nova Scotia, Collins 1885; Iceland, Faeroes, Scotland and northern Ireland, Schmidt 1909; North sea, Graham 1934; Norwegian waters, G. M. Dannevig 1908, A. Dannevig 1933 and Eggvin 1934; Spitzbergen and Bear island, Iversen 1934), but spawning may extend into June (off west Greenland, Jensen and Hansen 1931) or July (Danish waters, Poulsen 1931), or even through June and July into August (Canadian gulf of St. Lawrence, Dannevig 1919).

Considerable spawning is reported as occurring as early as December (parts of the gulf of Maine, Bigelow and Welsh 1925), and some may take place as early as November (Nantucket shoals, Schroeder 1930), or even September (Ipswich bay, Fish 1928). It is noteworthy that this autumn spawning does occur in some instances at relatively high temperatures and accordingly may be comparable to the spawning in warm water (8 to 15° C.) during late September to early December in the Halifax region (McKenzie 1934), to be dealt with in this paper. However, it has not been carefully investigated, nor has it been reported to be other than the early ripening of some of the more usual "runs" of fish, and is continuous with the winter spawning. This may not be true for the autumn spawning in very cold water (3 to 5° C.), reported around Newfoundland and Labrador by Nielsen (1894), Munn (1922), Frost et al. (1933) and Wilson and Thompson (1932, 1933 and 1934), or in the North sea (temperature 5° to 8° C.) by Fulton (1904) and Graham (1929). The usual temperature range for spawning of cod is from 4 to 6° C. with an extreme reported range of −0.3 to 8° C.

Kändler (1938) has recently reported spawning of cod in September in the southern part of the Baltic. His data are not very extensive, but it would seem that this is similar to what will be described in this paper, particularly because he notes a lower average number of vertebrae in the autumn spawners than in those spawning in the spring (52.0-52.1 vs. 52.5-52.6).

MATERIAL AND METHODS

The information about the fish has been obtained by experimental fishing, by examining commercial catches and by making inquiries of practical fishermen.

Jigs, line trawl, hand lines and gill nets of 13.7 and 15.6 centimetres stretched mesh measure, have been the implements used in capturing adult fish of this population. Only the jigs and gill nets have proved satisfactory, the latter being much the better.

Only small samples of the adult fish have been obtained each autumn because of the limited amount of fishing effort possible. However, gear has been fished at frequent intervals throughout the autumn and early winter months in Halifax harbour every year since 1933 inclusive. In this manner, obtaining only a few fish at a time, a small sample has been built up each autumn

and the whole season's collection has been treated as one sample. The St. Margaret bay information has been obtained through the examination of three to six commercial catches each autumn.

A macroscopic examination of the gonads indicated the sex of the adult fish.

The adult vertebral columns were prepared for examination by filleting or splitting and cutting open the air bladder. The urostyle has not been included in the vertebral counts which were made without the aid of magnification. Abnormal vertebral columns (less than three per cent were seen) have not been included in the calculations.

Eggs and larvae were secured with number 0 (bolting silk standard of mesh) marquisette nets approximately five metres long on metre hoops. The hauls, of fifteen minutes' duration, usually were made by towing the nets in a small circle with the engine going at a set slow speed. The actual speed of the boat was approximately 1.7 knots (3.15 km.) per hour but varied somewhat depending on wind and current. Hauls were made generally at 0 to 2 metre depths and 18 to 23 metre depths, where the total depth permitted, and on certain cruises near the bottom at stations of over 30 metre depths. The net was closed before bringing it to the surface on all tows made at other than the 0 to 2 metre depths.

The samples were preserved in water with 2 to 4% by volume of formalin (37% formaldehyde solution). The eggs were classified into stages of development numbered from I to IV, each stage representing three successive stages as defined by Meek (1924). The fry lengths and egg diameters were measured to the nearest tenth of a millimetre with a micrometer eye piece.

RESULTS

The Adult Fish and Fishery

From 1933 to 1938 it has been found that the autumn spawning cod have never arrived in Halifax harbour, from which the most of our data have been derived, earlier than September 20 or later than October 10.

During the early part of September the regular summer cod are caught by line trawl and hand lines in the outer part of the harbour (which is about 13 km. long excluding Bedford basin) chiefly, and their gonads are not more than half developed towards the spawning stage. At this time, practically no cod are to be found on the fishing grounds in the inner part of the harbour where cod are taken in the spring and early summer. Late in September a few cod having well developed gonads, but not quite "running", appear in the catches from the outer harbour. During recent years when this has happened cod gill nets have been set at the bottom on the inner harbour grounds with daily increasing success. In contrast to the behaviour of the resident cod, this spawning is quite near shore, the innermost of these grounds being within 1 km. of two islands and some of the steamer piers.

In St. Margaret bay, the fishermen at the mouth of the bay report that every year about September 20, they catch some cod full of spawn along with the smaller, sexually immature cod usual at this time of year. This lasts for only one to three days and they then say that these fish "have gone to the

head of the bay". At the head of this bay the line trawl fishermen make rather poor catches during the latter part of the summer, but as soon as any of these "spawn" fish are taken, the fishermen change over to gill nets set on the bottom as in Halifax harbour. These nets are usually set first about October 1 and fishing continues to be profitable until about the middle of November, at which time the weather usually is the prohibiting factor rather than lack of fish. In this inlet, too, the fishing grounds are very close to the islands and shores at the head of the bay.

Both in Halifax harbour and St. Margaret bay the water over the spawning grounds varies from as deep as thirty metres to as shoal as fifteen metres, and the bottom is very rough and rocky. A luxuriant growth of brown algae (*Desmarestia viridis*) covers the bottom on most of the known spawning grounds and fouls the gill nets badly.

Table I gives temperatures in Halifax harbour during the autumn and winter seasons. It can be seen that very striking changes take place from week to week during the autumn. These affect the spawning fish. Spawning takes

TABLE I. Temperatures taken in 30 metres of water just beside a spawning ground, Halifax harbour

Depth (m.)	Sep. 21 1938	Sep. 27 1938	Oct. 5 1938	Oct. 26 1938	Nov. 7 1938	Nov. 17 1938	Nov. 29 1938	Dec. 8 1938	Jan. 8 1935	Feb. 13 1937	Mar. 13 1935	Apr. 9 1935
0	17.0	12.9	13.2	9.7	10.0	6.8	8.7	7.0	2.0	1.0	−0.5	1.5
5	16.6	12.4	—	11.1	—	—	8.0	5.2	1.5	0.5	−0.8	0.2
10	15.4	10.2	13.4	11.8	11.8	8.8	8.0	4.8	2.4	0.5	−0.8	0.0
20	15.2	8.9	12.2	10.2	11.2	8.0	7.6	4.2	2.4	1.0	−0.4	0.0
30	13.7	5.2	10.4	5.8	10.2	6.5	6.8	3.3	2.3	1.2	−0.2	0.1

place on this ground (Mahars rock) in from eighteen to twenty-five metres. Sudden and severe drops in temperature bring about a cessation of spawning, and the fish either leave the grounds completely or remain quiet, for no fish are caught either with baits or gill nets at such times, nor are any newly spawned eggs obtained. An instance of this was observed in early October, 1934, when the bottom temperature dropped from 11.8 to 3.5° C. in six days. During this time and until the temperature had again risen to 8° C. no cod were captured anywhere in the vicinity of the shoal and the number of eggs obtained in the tows decreased to almost nil.

While in general the temperatures on these spawning grounds range from about 15° C. down to even 3° C. during the spawning season, to judge by the catches of fish, the cod are spawning in greatest numbers when the temperature is between 12° C. and 8° C. Surface temperatures as high as 19.0° C. have been recorded during this period.

During late December and until March the water temperatures in this region are much lower than during the autumn, hovering around zero Centigrade, as seen in table I.

During the autumn the salinity varies from 30.6 to 31.8‰, a variation

which is believed to be insignificant in its effect on the movements of these cod, though Bull (1938) has shown that cod will react purposefully to changes as slight as $0.2^0/_{00}$.

In collecting these fish, it was found, as is known to be the case with other species of fish at spawning time, that they fed but little, hence methods of capture not dependent on bait proved the most successful. In the examination of 156 stomachs during this spawning season, 86% were found to be empty and only 11 of the remainder contained more than a trace of food.

Throughout the summer, both sexes of the "resident" cod have been found to be present in almost equal proportions on all the fishing grounds in and off Halifax harbour. This also applies to those cod living off the harbour in the autumn. However, in six annual samples of autumn-spawning cod from the inner part of Halifax harbour, and in three from St. Margaret bay 77% of the 906 fish obtained were males. This was approximately the percentage found for both inlets and also for all years.

The mesh of the nets may exercise a selective action in favour of the males because they are smaller and gill more readily. In the 1936 sample from Halifax harbour the average weight of the males was 4.6 kg. (10.2 lb.) as compared with 8.9 kg. (19.7 lb.) for the females. The use of a net of larger mesh might increase the proportion of females found in the school, but the result of none at all being taken early and late in the season would probably not be altered.

At the beginning and end of the "run" each autumn, as is generally true for most fishes, the spawning school is composed almost entirely of males. However, from the middle of October until late in November, females are more numerous in the catches, but at no time have they ever made up as much as 50% of the catch.

The males thus arrive on the spawning grounds before, and remain after, the females, but their greater apparent abundance is no doubt due not only to

TABLE II. Lengths of the samples of adult autumn spawning cod, taken in October and November

Year	Total no.	Length frequencies in cm.												
		50 to 54	55 to 59	60 to 64	65 to 69	70 to 74	75 to 79	80 to 84	85 to 89	90 to 94	95 to 99	100 to 104	105 to 109	110 to 114
Halifax harbour														
1933	88	6	10	10	14	12	10	13	6	3	1	1	1	1
1934	68	2	2	13	16	7	7	6	7	6	2	0	0	0
1935	131	1	6	28	32	18	14	11	10	7	3	1	0	0
1936	135	2	4	20	19	34	17	17	9	8	3	1	0	1
1937	95	1	3	9	14	25	19	15	7	1	0	1	0	0
1938	123	0	0	2	8	28	31	22	20	9	2	1	0	0
St. Margaret bay														
1936	104	2	7	21	28	15	13	8	7	1	0	0	0	2
1937	96	0	0	4	6	21	21	28	10	3	1	2	0	0
1938	108	0	0	2	13	22	15	24	19	10	0	0	2	1

selective action by the nets, but also to the greater activity of the males at spawning time (which is true of most fishes) increasing the frequency of their capture.

These cod are peculiar not only in their season of spawning, but also in their possession of a lower average vertebral count than is known for any cod population on the whole Atlantic coast of North America.

In table II the length frequencies of various samples for the period 1933 to 1938, nine in all, are set forth, and it can be seen that these cod ranged in length from 50 to 114 cm., though few were below 55 or over 95 cm., and about 85 per cent were between 60 and 90 cm. in length.

In table III are given the vertebral counts for six yearly samples of autumn spawning cod from Halifax harbour and for three from St. Margaret bay, together with counts for three other samples of cod from the Halifax harbour region.

TABLE III. Vertebral counts of cod from the Halifax - St. Margaret bay region

Year	Sample number	No. of vertebrae						No. in sample	Average number	P.E.
		50	51	52	53	54	55			
Autumn spawning fish										
Halifax harbour										
1933	1	3	35	42	7	1	—	88	51.64	.053
1934	2	2	23	36	7	—	—	68	51.71	.057
1935	3	5	40	56	13	—	—	114	51.68	.046
1936	4	5	42	73	15	—	—	135	51.73	.041
1937	5	2	25	64	4	—	—	95	51.74	.039
1938	6	6	30	63	9	—	—	108	51.69	.046
St. Margaret bay										
1936	7	7	26	42	5	1	—	81	51.59	.058
1937	8	3	27	48	18	—	—	96	51.84	.052
1938	9	4	38	74	7	—	—	123	51.68	.038
Resident fish										
Halifax harbour mouth										
Autumn 1934	10	1	3	14	35	19	—	72	52.94	.069
Halifax harbour										
Summer 1934	11	—	4	36	41	30	1	112	52.89	.056
Mixed fish										
Halifax harbour mouth										
Spring 1935	12	2	21	46	24	10	1	104	52.21	.064

Adopting a value of three times the probable error of the difference as a significant difference in the means (e.g. maximum difference, $51.74 \pm 0.039 - 51.64 \pm 0.05 = 0.1 \pm 0.066$), it is found that all the autumn Halifax harbour samples (numbers 1 to 6) fall within this limit. The differences in these means are well within the limit set (in example above, $3 \times 0.066 > 0.1$), hence the

vertebral counts do not show that the schools of cod which come to spawn in Halifax harbour each autumn are significantly different from year to year.

In the same way ($52.94 \pm 0.069 - 52.89 \pm 0.056 = 0.05 \pm 0.089$ and $3 \times 0.089 > 0.05$) the school of resident cod found at the mouth of the harbour in the autumn (sample 10) does not differ from that found well up in the harbour during the summer (sample 11).

However, a comparison of these two populations (autumn spawners and resident fish) shows that the difference ($52.89 \pm 0.056 - 51.69 \pm 0.046 = 1.2 \pm 0.072$) between the means of these two populations is about fifteen times the probable error of the difference in the means. Thus, it may be concluded that the difference in the means is not due to chance and that the populations are distinctly different.

The fish that on the basis of vertebral count belong to the population of resident fish are spread out over the harbour during the summer, and concentrate late in the summer at the harbour mouth where the water is deeper and cooler. As a result of this concentration, there are few, if any, of these fish caught within the harbour late in the summer, but in early autumn the harbour is invaded by the population of autumn spawners which pass in through the concentration of resident cod at the mouth. At this time the water temperatures in the inner harbour (table I) are quite high.

After spawning is over, usually early in December, the "spent" cod gradually move out to the mouth of the harbour where they remain until April–May mixed with the resident fish. Sample 12 in table III shows that the range of the vetebral counts of these fish at the harbour mouth during the late winter and early spring covers that of both populations. Usually, by early May, the autumn spawning population leaves the region altogether and the vertebral count of codfish sampled anywhere in the shore waters of the Halifax vicinity is as shown for sample 11.

A comparison of the vertebral counts of the autumn samples of 1936, 1937 and 1938 from St. Margaret bay with those spawning in the autumn in Halifax harbour does not show these two spawning groups to differ significantly from each other. The St. Margaret bay samples, however, show considerable variation in their averages in contrast to those from Halifax harbour. This may be due to the fact that the former were caught commercially and in some cases were eviscerated at sea, which did not permit what was done at Halifax, the exclusion of fish that were not spawning.

The Eggs

After finding spawning cod in the harbour at Halifax in the autumn of 1933, plankton tows for eggs and larvae were commenced at a series of stations which were established. From the results obtained during the succeeding year, key stations were selected to which the tows were in general restricted in subsequent years.

Figure 1 shows the average number of eggs obtained per fifteen minute tow at weekly intervals during 1934 to 1936 inclusive. This station was located just off Mahars rock, Halifax harbour, close to the chief spawning ground.

From this, it is seen that no eggs were obtained previous to the week of September 24 and only small numbers up to about October 10. Spawning was quite intensive generally from then until mid-November. Few, if any, eggs were found after the first part of December, and none from January to March in tows taken during the years 1931 to 1938 inclusive, the resident cod beginning to spawn only by April.

During late December, January, February and March cod eggs are practically never obtained in any of the tows taken in or off Halifax harbour, but from then on until spring the native cod carry out their spawning.

The depth at which the majority of these autumn cod eggs occur is shown by data from tows made usually at two standard depths and near the bottom

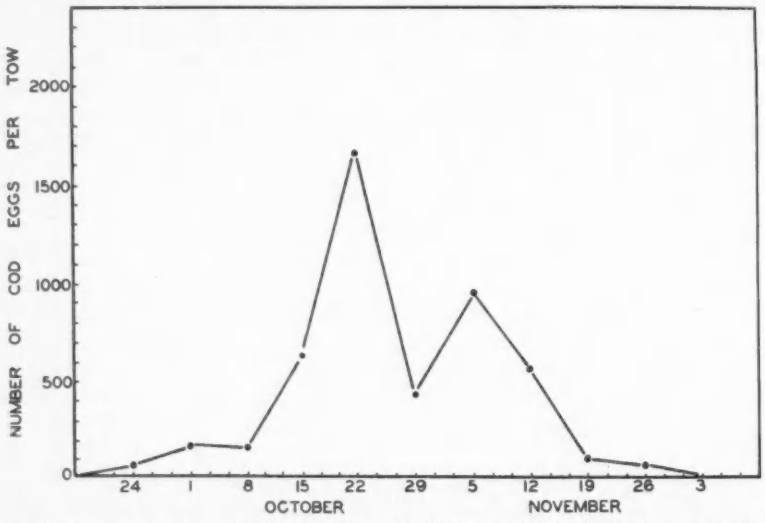

FIGURE I. Average number of cod eggs per tow off Mahars rock, Halifax harbour, 1934 to 1936 inclusive, during the autumn weeks indicated.

on a survey in 1935. The data for nine stations extending over the coastal waters from St. Mary bay to Chedabucto bay are given in table IV.

Although some variation from station to station was found, the data show that on the whole the eggs are near the surface rather than near the bottom.

The greater percentage of the younger eggs is found in the upper layers of the water. After allocating 44,405 eggs to their various developmental stages, i.e., I to IV, as outlined previously, it has been found that 78% of the 34,019 eggs in stage I, 74% of the 7,601 eggs in stage II, 61% of the 2,341 eggs in stage III and 28% of the 444 eggs in stage IV, were taken in tows made at the 0 to 2 metre depth. The remainder were taken at the 18 to 23 metre depth. Only eggs obtained at stations where both tows were taken enter into these calculations. This is shown graphically in figure 2. It may be concluded that during the earlier stages of development the eggs float nearer the surface than they do just preceding hatching.

The average size of these autumn spawned eggs—diameters of 7,251 eggs measured with a micrometer eyepiece—has been found to be 1.36 mm. with a range of 1.12 mm. to 1.55 mm. On the other hand, 3,145 eggs from winter and early spring spawning cod, measured in the same manner, gave an average of 1.5 mm. and a range of 1.3 mm. to 1.7 mm. in diameter. The latter eggs were collected about southwestern Nova Scotia in 1932 (McKenzie 1934) at a time of year when the water temperatures are very much lower than they are at the time of autumn spawning. This inverse relationship between egg size and water temperature was found by Fish (1928) in the Grand Bank vicinity and also in Massachussetts bay, as well as by other investigators both in nature and in experiments.

TABLE IV. Eggs taken at stations with depths greater than 30 metres

Location	0 - 2 m.	18 - 23 m.	30 m. plus
2 m. S.E. Cape Roseway.....	5	0	5 (33-37 m.)
9 m. S.E. " "	14	1	0 (40-45 m.)
Mouth Mouton bay.....	87	3	83 (30-35 m.)
Mouth St. Margaret bay			
West side.....	1,556	6	34 (40-45 m.)
East side.....	256	51	66 (33-38 m.)
Mid. St. Margaret bay			
West side.....	420	267	11 (40-45 m.)
East side.....	571	421	123 (44-48 m.)
3 m. off Betty island.....	142	49	24 (65-70 m.)
Off Queensport			
Chedabucto bay.....	83	24	5 (35-40 m.)
	3,134	822	351

As shown previously, there is a great difference in the total numbers of eggs obtained of the four different developmental stages. During the autumns of 1934, 1935, 1936 and 1938 tows were taken at both 0 to 2 metres and 18 to 23 metres at one hundred and sixty-four stations. Twenty-eight of these were in Halifax harbour or vicinity, twenty-five in St. Margaret bay and one hundred and eleven at points along the shore (none off in water of over 75 metres depth) from the bay of Fundy to Cape Breton island. Of the latter, sixty stations yielded no eggs or larvae in the tows. These tows were taken at numerous points all over Halifax harbour and St. Margaret bay, as well as along shore outside of these inlets. Most of these shorewise tows were taken to the southwest of these inlets, since the general drift is considered to be in this direction. The remainder were taken at other points along the shore.

Of the 51,954 eggs obtained, 41,164 or 79% were in stage I of development, 7,794 or 15% in stage II, 2,539 or 5% in stage III and 455 or 1% in stage IV which just precedes hatching. Only 235 cod larvae were obtained. Thus, only about 0.5% of all stages were larvae.

Eight of these tows were taken in September, fifty-eight in October, eighty-five in November and thirteen in December. Since, as shown previously, spawning

is well under way by the third week of October and since Meek (1924) has shown that at 5.5° C. cod eggs hatch in about twelve days (17.2 days at 6° C. according to Bonnet, 1939), it is to be expected that the November tows should have taken large numbers of well developed eggs and larvae. However, such was not the case.

Many of the tows were taken in the vicinity of spawning grounds where probably more young eggs—though it depends on the drift away—would be obtained, but tows were also taken at distances from these grounds where the eggs should have been in later stages of development as they drifted away from the centres of production. However, many fingerling cod are found in Halifax and St. Margaret bays each spring, hence many eggs must remain in these regions, and consequently the numerous tows taken here during the autumns should reveal the degree of survival, to some extent at least, in these inlets.

In table V, station 1 is in the harbour, 2 at the west side of the mouth of the harbour, 4 about eight kilometres southwest along shore and two off, and 3 about the same distance southwest but ten kilometres off shore. The eggs

TABLE V. Eggs taken in the Halifax harbour region, November 10, 1935

Stage	0 - 2 metres				18 - 23 metres			
	Station				Station			
	1	2	3	4	1	2	3	4
I	591	33	8	26	62	4	4	4
II	227	20	7	68	31	4	2	9
III	45	7	6	16	15	5	0	7
IV	7	2	4	2	0	0	1	1

are seen to diminish at both depths from stations 1 to 3, but increase again at 4. This last is due to additional egg production near 4. Dissemination of the eggs may cause the decrease from 1 to 3.

Table VI shows a similar picture obtaining in St. Margaret bay. Station 2 at the mouth of the bay is located on the western side and most of the eggs are seen to apparently leave at this side. No spawning takes place in Mahone bay and as the eggs pass the mouth of this bay a great reduction in their numbers is seen to have taken place, so much so, that by the time the Lunenburg region is reached, about twenty-five kilometres southwestward along the shore, no eggs were found.

Dissemination could cause the reduction in number of eggs per tow as the distance from the source of production increases, but some disaster must befall the eggs to have them all vanish at the height of the spawning season in a distance of twenty-five kilometres.

Vagaries in the drift and cessation of spawning for a few days might possibly account for finding no eggs off Lunenburg on November 4, 1935, but when all tows are considered for the three autumns, it is found that at no station or at no depth did the number IV stage eggs make up over 17% of the total eggs in

the tow, and in quite a number of instances, as for example off Lunenburg in 1935, no eggs were found at all. If there is a high degree of survival to the hatching stage in this spawning, at least some tow, during all the collections, should have yielded either all larvae, the majority of the eggs in stage IV or a combination of the two. However, since no such tow was found, it is believed that the very low percentage survival derived previously from the whole collection of about 52,000 eggs gives a fair indication, though somewhat exaggerated due to a high proportion of the stations being close to spawning grounds, of what generally

TABLE VI. Eggs taken in the St. Margaret bay region, November 4, 1935

District	Mid bay		Mouth of bay		Mouth of Mahone bay		Off Lunenburg	
Station no.	1	2	1	2	1	2	1	2
0 to 2 metres								
Stage I	510	280	199	1,306	45	146	0	0
" II	42	119	45	245	71	21	0	0
" III	15	20	11	5	7	6	0	0
" IV	4	1	1	0	0	2	0	0
18 to 23 metres								
Stage I	309	171	37	6	10	0	0	0
" II	41	55	4	0	15	0	0	0
" III	63	35	9	0	2	0	0	0
" IV	8	6	1	0	0	0	0	0

occurs in this autumn spawning. This is in agreement with Dannevig (1919) who found that the number of cod larvae in the gulf of St. Lawrence was remarkably small. Also Walford (1938) found that in 1931 the cod-haddock eggs were not carried away from Georges bank to any extent, and that the May haddock larvae were largely the result of April spawning, with the larvae much less abundant than the eggs. However, Hart and Tester (1934) found for the Pacific herring that the greatest mortality occurred after hatching, between the larval and the adult stages.

Among the possible lethal factors, three appear to be more important. Firstly, the great numbers of eggs showing no development may be infertile. While they may have been newly fertilized, such eggs were found in all tows, and, since considerable cell development occurs within one day of spawning, even at 5.5° C. and much more so at the temperatures found over these autumn spawning grounds, it is believed that such would have occurred by the time the eggs had risen to the upper layers where the tows were made. Since infertile cod eggs remain suspended for about two days according to Dannevig (1919), it is tentatively concluded that these were infertile eggs. The comparatively small concentrations of cod on these spawning grounds, as compared to the winter spawning grounds, may possibly be responsible for this.

Secondly, Leim, in an unpublished report, has shown that in the Gloucester (Massachussetts) hatchery the best hatch from winter spawned cod eggs is obtained by keeping them between the temperatures of 2° C. and 8° C. Johansen

and Krogh (1914) placed the upper hatching limit at 10.2° C. Thus, unless the autumn spawned cod eggs have a higher optimum incubation temperature than these winter spawned eggs, the temperatures are unfavourably high for them during the greater part of the autumn season.

Thirdly, Rollefsen (1930 and 1932) found that almost 90% mortality resulted from dropping cod eggs in the early stages of development a short distance, in a water-drop, on tightly stretched silk. Shaking them moderately in a glass of water produced the same results, and he suggested that wave action might produce similar shock. If such be the case, these autumn spawned eggs may suffer great reduction in numbers as a result of being released close inshore in the vicinity of the "breakers" and at a time of year when violent storms and strong onshore currents are quite frequent.

The Larvae

The cod larvae secured in these collections are not heavily pigmented and apparently conform to Dannevig's (1919) warm-water type.

Most (81%) of the 235 larvae taken during this investigation were captured in the 18 to 23 m. haul, but as is shown in the following tabulation, this is a result of taking most of the hauls during the daylight hours:

Time	No. of larvae	Depth of haul	
		0 - 2 m.	18 - 23 m.
Day 8.00 a.m. - 4.00 p.m.	196	10%	90%
Night 4.01 p.m. - 7.59 a.m.	39	69%	31%

A movement of the young cod in relation to the intensity of daylight is clearly indicated and such an interpretation of the results is supported by the observations of Dannevig (1932) who found that cod fry were attracted by moderate light but repelled by strong light. Other species of young fishes have been found to exhibit this same characteristic.

The majority of these larvae, measured with the micrometer eyepiece, were between 3.8 mm. and 4.9 mm. in length, though several were almost 8.0 mm. in length. Table VII gives some details regarding the place and time of capture of these larvae, together with their size which is shown to increase on the average month by month.

Spawning Regions

Surveys for autumn-spawned cod eggs have covered the area from and including the bay of Fundy to and around Cape Breton island. Because of distances involved, no extensive studies of spawning regions other than by these egg surveys have been carried on except in Halifax harbour and St. Margaret bay. Figure 3 shows this Nova Scotian area and indicates the positions of the stations, except those in Halifax harbour and St. Margaret bay.

In surveying this region in the autumn, tows have been taken in all the bays and places which at all resembled the conditions obtaining in Halifax harbour and St. Margaret bay. Besides this, tows have been made at stations along the outer coast of Nova Scotia extending off from shore in series for distances of forty to eighty kilometres. Cod eggs have been found at a number

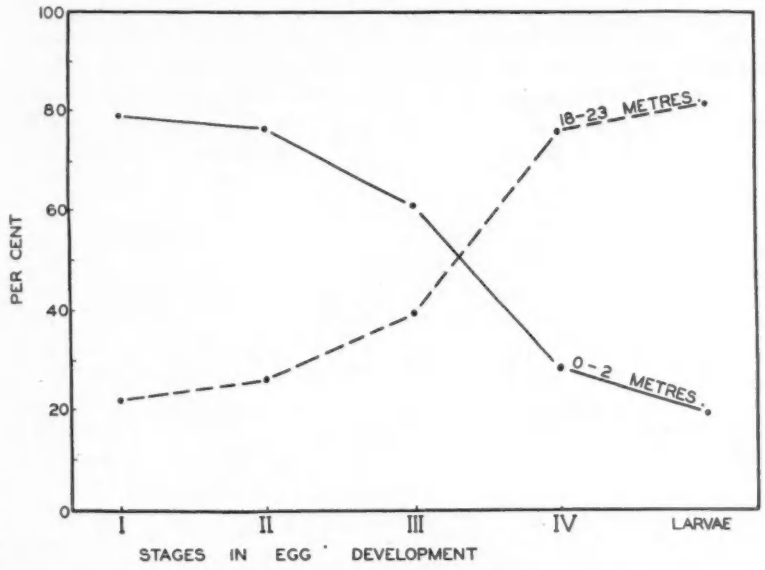

FIGURE 2. The percentage of the different development stages of eggs and the young larvae at the two different depths.

TABLE VII. Cod larvae obtained in the various districts from 1931 to 1938

Length (mm.)	October					November					December			
	3.0 to 3.9	4.0 to 4.9	5.0 to 5.9	6.0 to 6.9	7.0 to 7.9	3.0 to 3.9	4.0 to 4.9	5.0 to 5.9	6.0 to 6.9	7.0 to 7.9	3.0 to 3.9	4.0 to 4.9	5.0 to 5.9	6.0 to 6.9
Halifax harbour 1931-1938.....	40	20	..	..	..	28	19	5	1	1	..	1	1	..
St. Margaret bay 1935-1936.....	..	..	..	..	..	16	41	12	1	1	..	..	..	..
Halifax harbour to St. Margaret bay 1935-1937.....	..	..	..	..	..	35	38	13	1	..	..	..	..	..
East of Halifax harbour 1935 and 1937...	8	4	..	..	..	1	9	2	3	..	..	..	..	..

of stations along the shore and also at some of the innermost stations in the various series, but never at any of the outer stations of these series.

Also a few tows, taken in the autumn in the offshore bank regions, have not yielded any cod eggs. To further substantiate this absence of cod eggs in outer waters reports from the offshore fishing fleet indicate that there are no ripe cod on the offshore grounds during this period. Autumn spawning of cod seems thus to be confined to inshore waters.

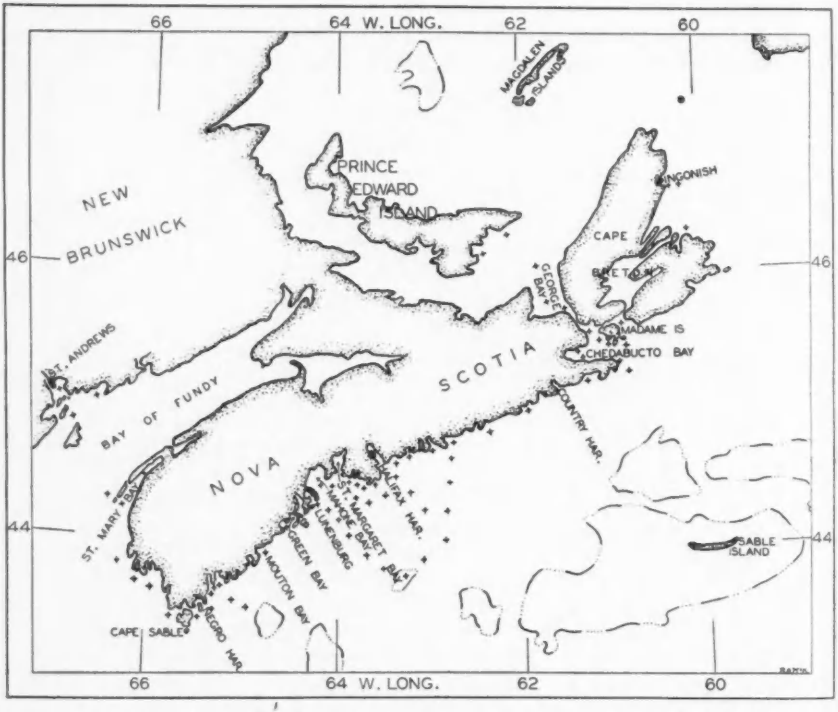

FIGURE 3. The Nova Scotian area showing station positions other than those in Halifax harbour and St. Margaret bay.

In the bay of Fundy, no spawning has been found to occur with the exception of a little in St. Mary bay in certain years. A few eggs are usually found around Negro harbour and Green bay, but St. Margaret bay and Halifax harbour show by far the greatest production, as many as two to three thousand eggs per fifteen minute tow with a metre net being frequently obtained. In certain years also, quantities of cod eggs are found off Country harbour, in Chedabucto bay and around the harbours at Ingonish, but at other points around outer Cape Breton and in the general George bay to Prince Edward Island area, no cod eggs have been found in the autumn.

DISCUSSION

The facts advanced make it reasonably certain that there is a spawning of cod in Halifax harbour and in certain other Nova Scotian inlets every autumn and that this is distinct in time from the usual winter-spring spawning that occurs in this as in other regions of the North Atlantic in which the cod is found. That the spawning fish are distinct in character is evidenced not only by their spawning at a time when the temperature is considerably higher than it is for the usual spawning, but also by the spawning taking place toward the heads of inlets rather than out to sea and by the fish themselves possessing a comparatively low vertebral count, which, in accordance with Schmidt's (1930) findings, would be expected as a result of the high temperature (17 to 5° C.) at which the eggs develop.

SUMMARY

The native codfish move to the mouth of Halifax harbour by September and a school of spawning cod enters about the first of October, proceeding to rocky spawning grounds near the head of the harbour and remaining there until early December. From then until April they stay in the deep water at the mouth of the harbour.

These fish, as caught during 1933 to 1938 with gill nets of 13.7 and 15.6 centimetre mesh, are predominantly males. Netting proves most successful since they do not feed. In contrast to the "resident" fish which have an average vertebral count of 52.9, these possess an average vertebral number of 51.7. Few females are caught at the beginning and end of the "run".

In size, these fish range from 50 cm. to 114 cm. with 85% being between 60 cm. and 90 cm. in length.

St. Margaret bay samples give approximately the same results.

Most of the spawning takes place between late September and early December in temperatures of 8° C. to 12° C. Below 8° C. spawning usually ceases. The temperature in the upper layers where the eggs float ranges from about 17° C. early in the season to 5° C. by early December.

Plankton tows at various levels showed that three-quarters of the eggs floated between the surface and a depth of fifteen metres, and only 8% were found at depths greater than 30 metres. In fact, about 75% of the eggs in stages I, II and III were taken at the surface. However, 28% of the eggs in stage IV were taken at the 18 to 23 metre depth.

These autumn spawned eggs are smaller than the winter—spring spawned eggs of western Nova Scotia—average diameter of 1.36 mm. compared to 1.50 mm.

Eggs and larvae were not obtained in tows made at any great distance from centres of production, and only 1% of all the eggs obtained was in the stage just preceding hatching. A high mortality thus seems apparent.

The larvae, showing a comparatively light warm-water type of pigmentation, were taken mostly at the 18 to 23 metre depth during the day, but in increased numbers near the surface at night. Their average length is shown to increase from October to December.

Surveys during the autumn in the bay of Fundy and along the outer Nova Scotian coast to Cape Breton by means of plankton tows show that the main centres of autumn Cod spawning are in St. Margaret bay, Halifax harbour and Chedabucto bay. The summer to early autumn spawning in the gulf of St. Lawrence is not, as yet, thoroughly investigated, nor has its relation, if any, to this spawning been determined.

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Le Poisson Frais
II. Le Rôle du pH sur le Développement des Bactéries

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RÉSUMÉ

Les bactéries du poisson sont très sensibles aux variations de pH à 0°C. Leur croissance est d'autant plus retardée que le pH est plus bas. Même leur nombre diminue à pH 5.0 et moins, pendant les premiers jours.

Il en résulte un retard analogue dans la formation d'azote volatil total et triméthylaminé avec l'abaissement de pH. La teneur de ces bases reste constante à pH 5.0, durant les vingt-cinq premiers jours de conservation à 0°C. La décomposition du poisson se trouve alors retardée, grâce à une diminution de l'activité microbienne.

INTRODUCTION

A cause de la faible consistance de sa chair, le poisson est un des aliments les plus périssables. C'est pourquoi un grand nombre de travaux ont été faits dans le but de trouver le préservatif idéal qui ne serait nullement dommageable à l'organisme humain à la concentration employée et qui, en même temps, empêcherait tout développement bactérien ou, du moins, le retarderait d'une façon appréciable.

A date, ce préservatif idéal n'a pas encore été trouvé. Il existe bien quelques substances telles que l'acide borique et ses sels, l'acide benzoique et ses sels, dont l'efficacité a été mise hors de doute par plusieurs auteurs, mais dont l'emploi est limité par l'incompatibilité gastrique de tout produit antiseptique contenu dans les aliments (Buzzo et Carratala 1932, Segalis 1934). Au cours d'une étude sur l'efficacité de ces substances, nous avons remarqué que leur action était beaucoup plus prononcée en solution acide, comme d'ailleurs l'ont démontré Fust (1929), Kuroda (1926), Goshorn, Degering et Tétrault (1938) et, tout dernièrement, Fellers et Harvey (1940). Les résultats obtenus nous ont conduit à étudier l'influence seule du pH sans préservatif, sur la conservation du filet de morue. Nous avons ainsi prouvé dans une première publication (Nadeau 1939) que, par augmentation de l'acidité de la chair de morue avec des acides organiques (citrique, lactique), sa durée de conservation était de beaucoup augmentée.

La formation de triméthylamine dans le jus musculaire ou les filets, maintenus à 0°C., était presque nulle même après trente jours, à pH inférieur à 5.0.

Nous complétons ce travail cette fois en étudiant l'influence de l'abaissement du pH, non seulement sur la formation de triméthylamine mais aussi sur la formation d'azote volatil total et sur le développement des bactéries dans la chair de morue, en relation avec sa conservation.

PARTIE EXPÉRIMENTALE

Mode d'opération

Pour plus de facilité, nous nous sommes servi de jus musculaire extrait par pressage de la chair du poisson. La méthode employée est celle décrite par Nadeau (1939). Une première série d'expériences est faite avec l'acide citrique, une autre avec l'acide lactique et une troizième avec l'acide tartrique, tous à la même concentration. Les différents pH, vérifiés à l'électrode de verre, sont obtenus par addition de ces acides en quantités différentes suivant le pH désiré. Il faut généralement 0.3 pour cent d'acide pour abaisser le pH à 5.0, et 0.6 à 0.7 pour cent pour obtenir pH 4.2. Dans chaque cas, le jus musculaire est porté à deux fois son volume, après acidification, afin de rendre comparables les différents résultats.

Sur chaque prise d'échantillon, la numération des bactéries a été faite après six jours d'incubation à 25°C. sur agar contenant 1.5 pour cent de sel. Trois pétris ont été ensemencés pour chaque dilution suivant la technique ordinaire. En même temps, l'azote volatil total et triméthylaminé a été dosé selon la méthode mise au point par Beatty et Gibbons (1936).

Résultats

La figure 1 nous indique les résultats obtenus avec l'acide citrique. On voit clairement sur ce graphique l'influence du pH sur la conservation du poisson. L'azote volatil total et triméthylaminé dans l'échantillon témoin (pH 6.6 au début) augmente rapidement; au bout de seize jours la décomposition du poisson est déjà assez avancée. Mais plus le pH baisse, plus la décomposition est retardée. A pH 5.0 et 4.2, les bases aminées ne commencent à augmenter qu'après trente jours.

Le pH a aussi une influence considérable sur la multiplication des bactéries. On voit par la même figure que l'abaissement de pH retarde leur développement. Au début, le nombre des bactéries augmente très peu et même diminue pour les pH les plus bas où l'augmentation des bases aminées est pratiquement nulle. Au bout de cinq à six jours, les bactéries se développent rapidement pour pH 6.0 et plus, tandis qu'à pH 5.0 et 4.2, l'augmentation est peu importante. Dans la suite, elles commencent à se multiplier, mais toujours plus lentement que dans le premier cas.

Les résultats obtenus avec l'acide lactique (fig. 2) sont sensiblement les mêmes qu'avec l'acide citrique. On voit de nouveau une différence marquée entre les courbes de pH 6.0 et celles de pH 5.0. Le graphique des analyses bactériologiques donne encore une meilleure idée du phénomène que le précédent. Le nombre des bactéries diminue, pendant les premiers jours, dans les milieux tamponnés à pH 5.0 et 4.2, pour ensuite augmenter toujours plus lentement que dans le témoin et le milieu à pH 6.0.

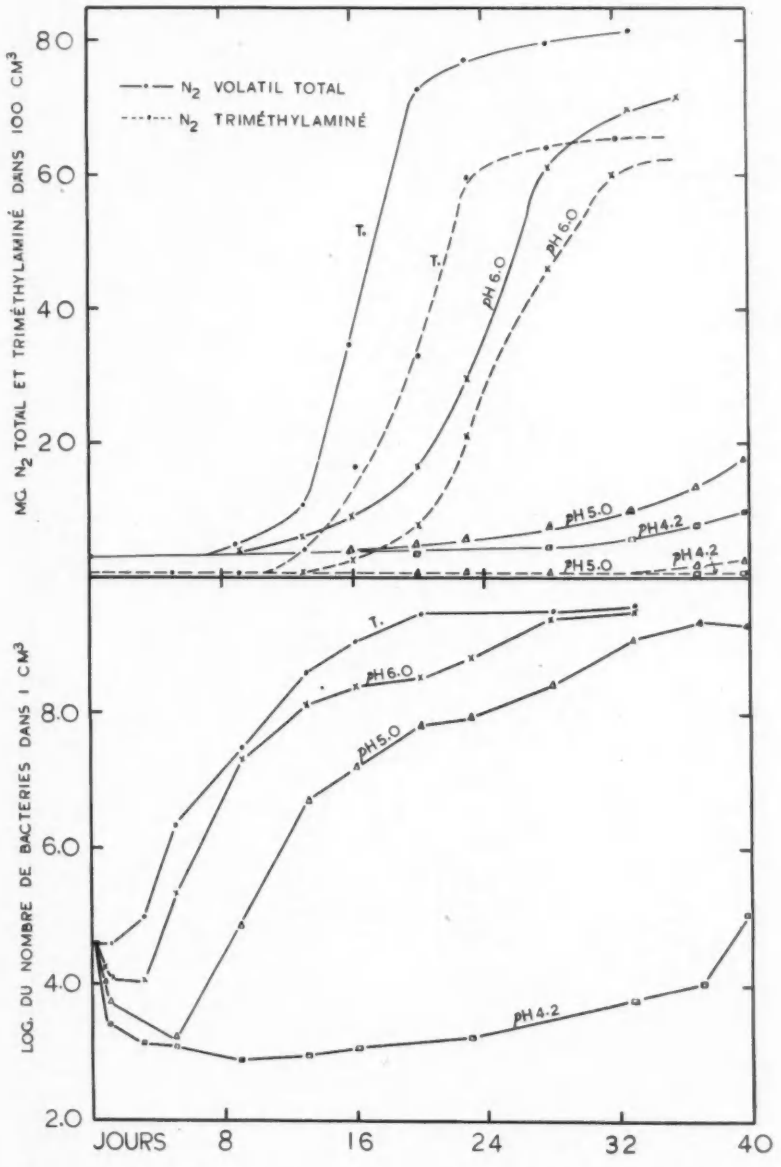

FIGURE 1. L'effet du pH sur le développement microbien et sur la formation de N_2 volatil total et triméthylaminé dans le jus musculaire tamponné avec acide citrique à 0°C.

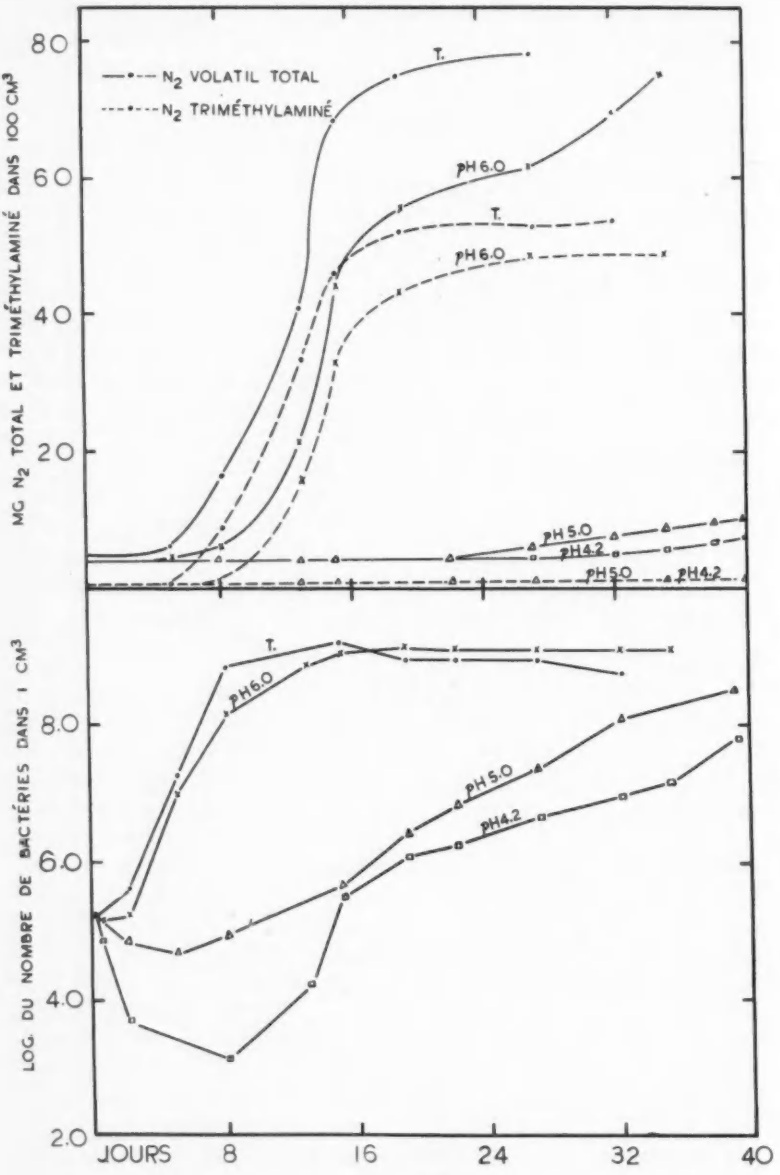

FIGURE 2. L'effet du pH sur le développement microbien et sur la formation de N_2 volatil total et triméthylaminé dans le jus musculaire tamponné avec acide lactique à 0°C.

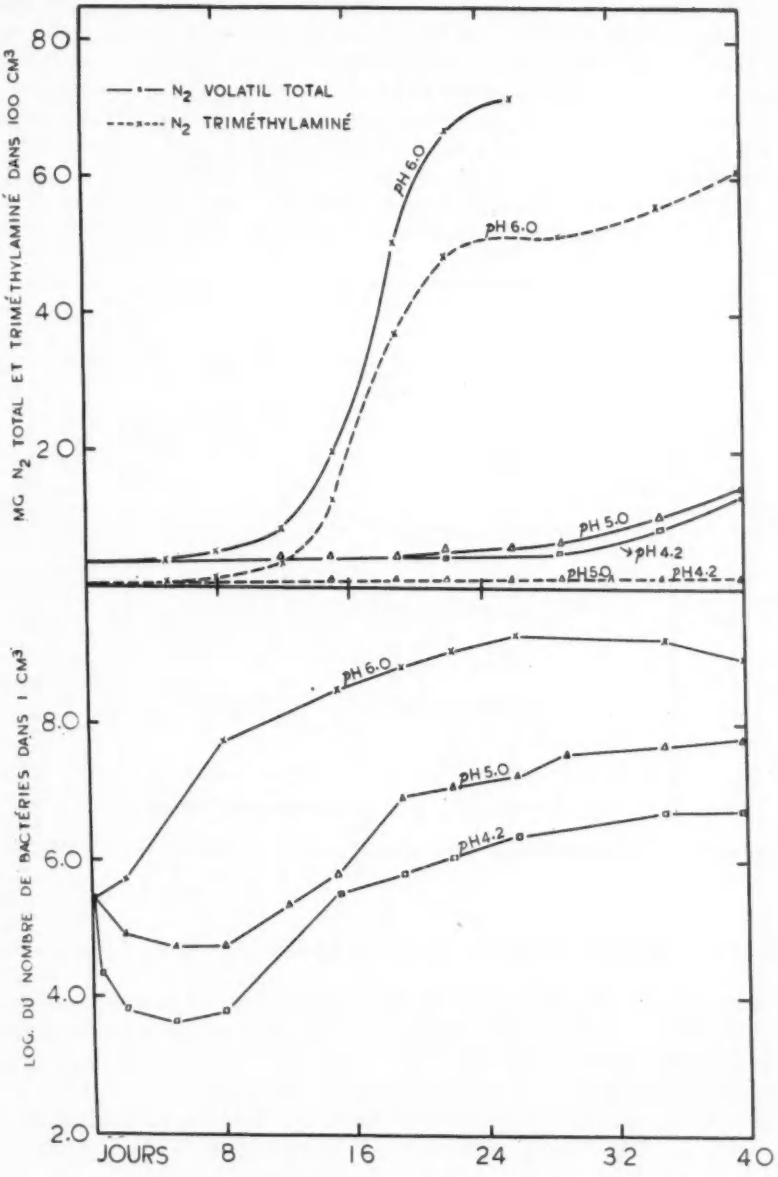

FIGURE 3. L'effet du pH sur le développement microbien et sur la formation de N_2 volatil total et triméthylaminé dans le jus musculaire tamponné avec acide tartrique à 0°C.

La figure 3 représente les résultats obtenus avec l'acide tartrique. Comme dans les deux cas précédents, il y a une différence marquée entre les courbes de pH 6.0 et celles de pH 5.0. Le nombre des bactéries diminue pour pH 5.0 et 4.2 pendant les premiers jours, tandis qu'à pH 6.0 leur nombre augmente rapidement. Il en est ainsi de l'azote volatil total et surtout triméthylaminé dont l'augmentation à pH 5.0 et 4.2 est pratiquement nulle pour les vingt-cinq premiers jours, mais rapide à pH 6.0. Il y a donc, d'après les figures 1, 2 et 3, un certain pH compris entre 6.0 et 5.0 où le retard dans le développement des bactéries a pour effet d'empêcher la formation de bases volatiles et surtout de triméthylamine.

Un fait intéressant à noter est l'élévation de pH dans les milieux où il y a

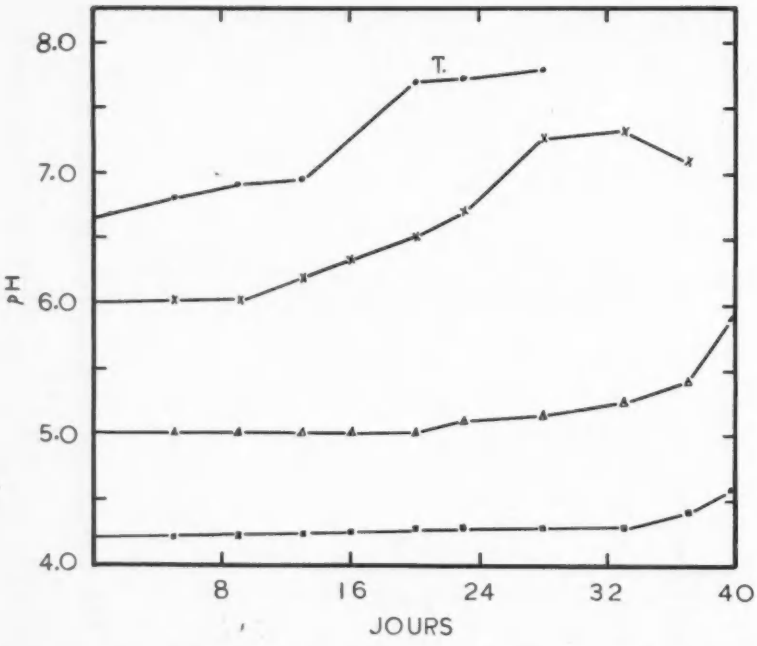

FIGURE 4. La variation de pH dans le jus musculaire tamponné avec acide citrique à 0°C.

formation importante de bases aminées comme l'a constaté Watson (1938). Il se produit pour le témoin (fig. 4) une augmentation rapide de pH entre quatorze et vingt jours, correspondant à une augmentation importante d'azote volatil total et triméthylaminé. De plus, le maximum de pH a lieu à peu près en même temps que le maximum du nombre de bactéries. Même constatation pour la courbe de pH 6.0. Par contre, dans les milieux où il n'y a pas d'augmentation rapide de bactéries, par le fait même, pas de formation importante de bases aminées, le pH reste constant jusqu'au-delà de trente jours.

Les mêmes conclusions peuvent être tirées des figures 5 et 6, qui représentent la variation de pH dans le jus musculaire tamponné respectivement avec l'acide

lactique et tartrique. Les maximums de pH correspondent, en général, au maximum du nombre de bactéries et, souvent, à une augmentation rapide d'azote volatil total et triméthylaminé. L'élévation de pH dans le poisson est donc un indice de sa décomposition. Se basant sur cette constatation, van Deurs et Hoff-Jørgensen (1936) ont préconisé une méthode sommaire et rapide de déterminer la fraîcheur du poisson.

ESSAI ORGANOLEPTIQUE

L'essai organoleptique a été fait sur des filets maintenus à 4°C. environ et tamponnés à pH 5.0, au moyen des acides citrique, lactique et tartrique, après

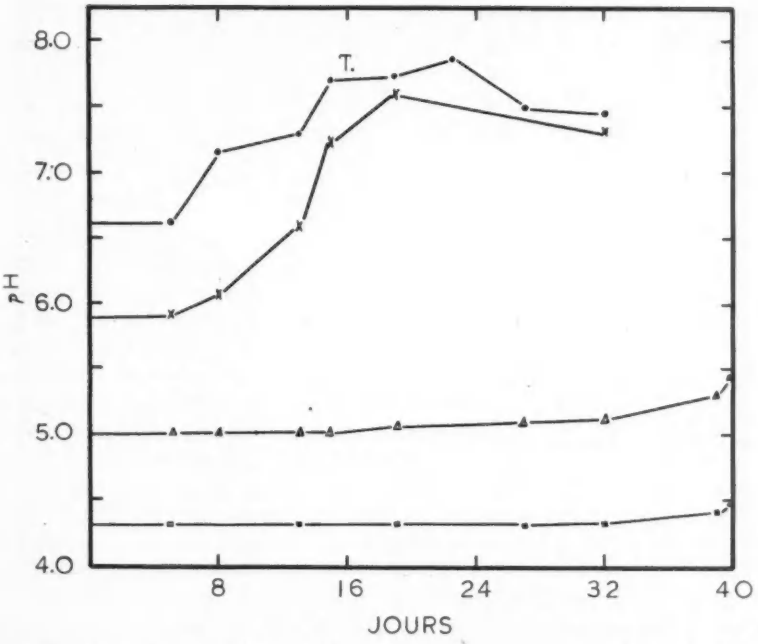

FIGURE 5. La variation de pH dans le jus musculaire tamponné avec acide lactique à 0°C.

un séjour de cinq, huit et dix-huit jours. Il n'y a dans aucun cas dégagement de mauvaise odeur à la cuisson. Ce sont les filets tamponnés à l'acide citrique qui ont la meilleure saveur et où le goût du poisson est le moins modifié. Dans un autre essai, des filets tamponnés à pH 5.0 avec le même acide et maintenus cette fois à 0°C. avaient encore bon goût après vingt-cinq jours. Dans tous les cas, les filets sont blanchis et gélatineux en surface.

DISCUSSION

Le résultat le plus intéressant à noter de ces expériences est la différence marquée dans l'allure des courbes obtenues entre le milieu à pH 6.0 et celui à

pH 5.0, qu'il s'agisse soit du développement des bactéries, soit de la formation de bases volatiles totales et de triméthylamine.

Tant que les bactéries n'ont pas atteint, semble-t-il, un certain potentiel, il n'y a pas formation de bases aminées. Mais aussitôt que, dans les conditions de nos expériences, les bactéries ont dépassé 10^7 par cm^3, l'azote volatil total commence à augmenter et dans la suite d'autant plus rapidement que le développement des micro-organismes est plus considérable.

Il en est de même au sujet de la formation de la triméthylamine. Mais dans ce cas, le potentiel semble être plus élevé (10^8 bactéries par cm^3). Ces faits se vérifient particulièrement bien pour le milieu tamponné à l'acide lactique (fig. 2).

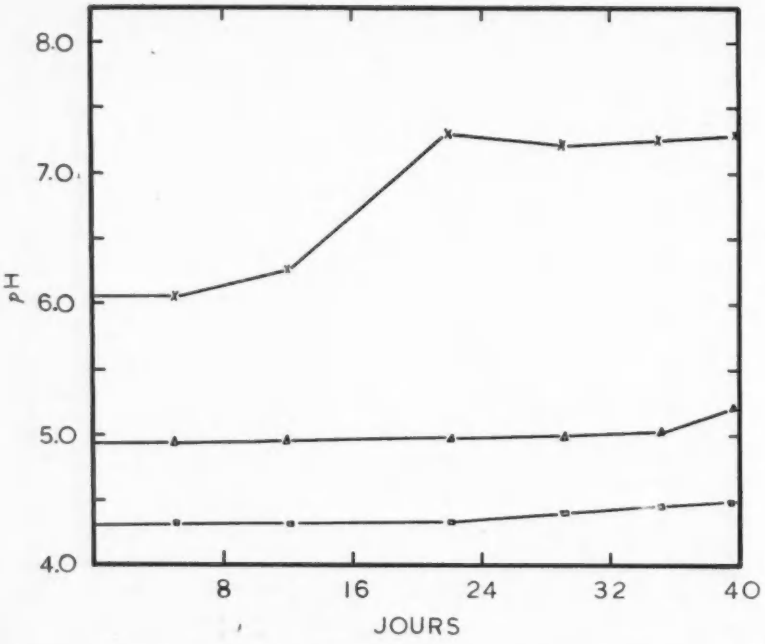

FIGURE 6. La variation de pH dans le jus musculaire tamponné avec acide tartrique à 0°C.

Il y a toutefois exception pour pH 5.0 où, dans certains cas, la quantité de triméthylamine trouvée reste pratiquement nulle même quand les bactéries ont dépassé 10^8 par cm^3.

A pH supérieur à 5.0, ces potentiels sont plus rapidement atteints, parce que l'activité des bactéries ne se trouve que peu réduite grâce à une faible modification du milieu. La mise en liberté de bases azotées, après quelques jours, a pour effet de neutraliser le tampon ajouté et de faire remonter le pH. Il s'en suit alors que les micro-organismes peuvent travailler dans un milieu plus favorable à leur développement, et la décomposition du poisson se trouve accélérée.

A pH 5.0 et moins, les conditions sont différentes, le milieu est fortement

modifié. Au début, le nombre des bactéries diminue sensiblement et leur activité se trouve par le fait même réduite. De plus le peu de bases formées n'est pas suffisant pour neutraliser le tampon ajouté, et le pH varie peu pendant les premiers trente jours. Ce n'est qu'après ce temps, quand le nombre des bactéries est devenu assez élevé (généralement 10^7 par cm^3), que le pH commence à monter. Mais dans ce cas, il n'y a que l'azote volatil total qui augmente, tandis que la triméthylamine reste pratiquement nulle. Les bactéries, probablement genre *Achromobacter* d'après Watson (1939), qui réduisent l'oxyde de triméthylamine, seraient pratiquement détruites à ces pH, ou leur pouvoir réducteur serait fortement diminué. Ce qui expliquerait l'absence de triméthylamine malgré que le potentiel ait atteint 10^8 bactéries par cm^3.

La courbe de l'azote volatil total suit plus exactement celle de l'augmentation du nombre des bactéries pour les différents pH que nous venons d'étudier. Le dosage de la triméthylamine comme test de la fraîcheur du poisson ne serait juste que dans le cas du poisson qui n'a pas subi de traitement. Dans les autres cas, celui des bases volatiles totales semble donner des résultats plus exacts.

CONCLUSIONS

L'abaissement de pH de la chair du poisson a pour effet de prolonger sa fraîcheur en retardant le développement des bactéries et, par le fait même, la formation de bases volatiles.

Dans le jus musculaire conservé à 0°C., le développement des bactéries qui est rapide à pH 6.0 et plus, est beaucoup retardé à pH 5.0 et 4.2. Même à ces derniers pH, leur nombre diminue pendant les premiers jours pour augmenter dans la suite, mais toujours plus lentement que dans le premier cas.

Dans les mêmes conditions, la formation d'azote volatil total et triméthylaminé diminue graduellement avec l'augmentation de l'acidité du milieu jusqu'à pH 5.0, où alors elle est pratiquement arrêtée pendant les vingt-cinq premiers jours. Après ce temps, l'azote volatil total commence à augmenter tandis que la quantité de triméthylamine reste pratiquement nulle, du moins pendant les quarante premiers jours.

L'avantage de ce procédé de conservation du poisson, susceptible d'application pratique, est dans l'emploi d'acides organiques (acides citrique, lactique et tartrique) que l'on rencontre à l'état naturel dans plusieurs aliments et qui sont nullement dommageables à l'organisme humain.

C'est, croyons-nous, un pas de plus vers le préservatif idéal qui, en plus d'être nullement dommageable, aurait pour but de rendre le poisson moins périssable, tout en lui conservant les propriétés qui caractérisent sa fraîcheur. Cette méthode a le désavantage de rendre les filets gélatineux en surface. En continuant ces travaux, nous croyons qu'il nous sera possible de remédier à cet inconvénient.

REMERCIEMENTS

Nous tenons à remercier Mlle Gertrude Roy pour l'aide qu'elle nous a apportée dans la réalisation d'une partie de ce travail.

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Food of the Rocky Mountain Whitefish *Prosopium williamsoni* (Girard)

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ABSTRACT

A study of 365 stomachs indicates the chief food to be aquatic insect larvae and other bottom forms. The type of food varies with locality, age, and season, depending on availability. The whitefish is of value as a game and food fish, but it may compete with trout and salmon for food, and may also destroy the eggs and young of these species.

INTRODUCTION

The Rocky Mountain whitefish, *Prosopium williamsoni*, sometimes erroneously called grayling, although prized by some as a sport and food fish, is regarded by others as a "coarse" fish unsuitable for human consumption and destructive to eggs and young of more valuable species. A determination of its true status depends on a complete knowledge of its interrelationships with other species. This requires a study of the importance of the whitefish as a food competitor and predator, as a game and food fish, and as a forage fish for trout and others. A knowledge of the feeding habits of the species is a direct contribution in this regard.

Very few detailed records of the food of *Prosopium williamsoni* appear in the literature. Numerous authors make the general statement that the chief food of this species consists of immature aquatic insects. Kemmerer and others (1924) and Neave and Bajkov (1929) present data which support these statements. Chapman and Quistorff (1938) examined 75 stomachs of this species and the closely related *Prosopium oregonium*, finding them to subsist almost entirely on bottom-dwelling insect forms. Snyder (1918) and Foerster (1925) record the presence of fish eggs in stomachs of the species. Munro and Clemens (1937) show that aquatic insects are the most important food, but also indicate that kokanee and coho salmon eggs may be taken in considerable numbers. In Okanagan lake (McHugh 1939) the stomachs of fish taken in the summer months contained chiefly Cladocera, with lesser amounts of aquatic insects.

MATERIAL AND METHODS

The present report is based on the examination of stomachs from 365 individuals taken from lakes and rivers in various parts of southern British

Columbia and Alberta. A large proportion of the material was collected by Dr. D. S. Rawson during the summers of 1936, 1937, and 1938 in National Parks in the Rocky Mountain region. The Cultus lake specimens were obtained in the course of sockeye salmon investigations by Drs. R. E. Foerster and W. E. Ricker, and a sample from the Elk river, a tributary of the Kootenay, was secured through the kindness of Mr. Wm. Stevenson of Michel, B.C.

The total volume of the contents of each stomach and the percentage volume of the various food organisms were estimated roughly with the aid of a binocular microscope. Ages of individual fish were determined by reading the scales. Stomachs which did not contain food were not included in drawing up the averages.

The data are presented in the form of tables. The numbers represent the average percentage volume of each organism present. Foods forming less than one per cent of the total volume are indicated by the symbol X. In many stomachs large quantities of mud, sand, gravel, and wood particles were present, but these were not considered in calculating the percentage volume of food organisms.

ACKNOWLEDGMENTS

Thanks are due to those who contributed material, particularly to Dr. Rawson for a great amount of original data. A part of the work was carried out at the University of British Columbia, and in this connection the facilities provided by Dr. C. McLean Fraser were much appreciated. The writer is also indebted to Dr. W. A. Clemens and other members of the staff of the Pacific Biological Station for much advice and criticism.

RESULTS

A summary of the food organisms of forty-six individuals in their first year is presented in table I. The stomachs of these fish contained bottom organisms almost exclusively, larvae and pupae of Chironomidae predominating and Ephemerida nymphs being frequent. Cladocera and other free-swimming aquatic forms appeared occasionally, and terrestrial insect material rarely. The young fish were taken in shallow water along the shores of lakes and streams and are apparently restricted in their feeding habits, taking food mostly at the bottom and only occasionally rising to feed on swimming or floating organisms.

Results of the stomach analyses of 319 individuals in their second year and older appear in table II. The food of the older fish appears to be more varied, and to depend to a considerable extent on availability and environment. In general the bottom feeding habit persists, the organisms most commonly found being the larvae and pupae of Chironomidae and other aquatic Diptera, larval Ephemerida and Trichoptera, and Gastropoda. Appearing less frequently in stomachs, but occurring in considerable quantities when present, are free-swimming and floating or flying organisms such as Cladocera and terrestrial insects.

The data also indicate variations in diet with locality, age, and season.

LOCAL VARIATION

Variations in food with locality are illustrated in table II. The most striking difference occurs between fish of lakes and of rivers.

The chief foods in waters such as Bow lake, lake Louise, and Waterton lake are larval Diptera, with Cladocera and other typically lacustrine forms playing an important role. Differences in diet between lakes are undoubtedly due to differences in the availability of the various organisms. In Okanagan lake, for instance, which is exceedingly poor in bottom fauna, Cladocera are taken in

TABLE I. Percentage occurrence by volume of food organisms in stomachs of *Prosopium williamsoni* in the first year.

	Bow lake	Lake Louise	Hector lake	Bow river near Exshaw	Average
Copepoda					
Cyclops sp.	—	12	—	—	1
Unidentified	—	—	—	X	X
Cladocera					
Alona rectangula	—	X	1	—	X
Alona sp.	11	5	—	—	3
Scapholeberis mucronata	—	32	—	—	3
Amphipoda					
Hyalella sp.	—	—	—	3	1
Hydracarina					
Unidentified	X	—	—	—	X
Insecta					
Chironomidae larvae and pupae	60	40	74	76	68
Simuliidae larvae	2	—	—	—	X
Unidentified Dipterous adults	7	—	—	—	2
Ephemerida nymphs	3	—	24	16	14
Plecoptera nymphs	6	10	—	—	2
Trichoptera larvae	4	—	—	—	1
Hemiptera	—	—	—	5	2
Homoptera	1	—	X	—	X
Psocidae	2	—	—	—	X
Hymenoptera	X	—	—	—	X
Unidentified terrestrial insects	3	—	—	—	X
Number of stomachs	12	4	15	15	

large numbers (McHugh 1939). In none of the other localities investigated is the Rocky Mountain whitefish so dependent on this type of food. The Ghost river reservoir and Kananaskis reservoir in the Bow valley are also poor in bottom fauna, and the presence of considerable quantities of terrestrial insects in the stomachs indicates that these fish will feed extensively at the surface on occasion.

The variety of organisms taken is generally much greater in rivers and streams than in lakes. Forms typical of stream bottoms, including Ephemerida

TABLE II. Percentage occurrence by volume of food organisms in stomachs of *Prosopium williamsoni* in the second year and older.

	Bow lake	Louise lake	Pipestone river	Lake Minnewanka	Cascade river	Third lake	Bow river: near L. Louise	Bow river: Bow river	Bow river: Lac des Arcs	Bow river: near Banff	Bow river: below Ghost dam	Ghost river reservoir	Kananaskis reservoir	Muskinonge lake	Knights lake	Pass creek	Waterton lake	Emerald lake	Elk river	Cultus lake
Protozoa																				
Arcella, etc.																				X
Gastrotricha																				
Unidentified																	X			
Annelida																				
Aquatic Oligochaeta	2											19	X						1	
Hirudinea																				
Unidentified leeches												3					5			
Ostracoda																				
Unidentified	X				X										5		X		X	
Copepoda																				
Cyclops viridis		2																		
Cyclops sp.		5															X			X
Unidentified	2																			
Cladocera																				
Daphnia longispina						33														
Daphnia pulex	4																			
Daphnia sp.		18				33											11			
Alona reclamuia		3																		
Alona sp.	4	15															X			
Eurycercus lamellatus					1															
Unidentified												X				X				
Amphipoda																				
Immature	X																			
Gammarus sp.					8												X			
Pyralella sp.					3															
Crangonyx sp.					3															
Unidentified					1				X								X			
Mysidacea																				
Mysis relicta																	X			
Hydracarina																				
Unidentified	X								X								X			X
Insecta																				
Orthoptera : Locustidae								36			40		5				X			
Neuroptera : Corydalis larvae													X							
Unidentified larvae													9						X	
Unidentified adults																	X			
Corrodentia: Psocidae																	2			
Ephemerida : Nymphs	15	1	70		100		25			2	X	19	11	80		25	X		1	
Plecoptera : Nymphs	5	1										X	X			15				
Hemiptera : Corixidae								X	12		X									
Notonectidae								21												
Saldidae	X																			
Lygaeidae	2																			
Unidentified										X	7									
Homoptera : Aphididae											2									
Cercopidae																	X			
Cicadellidae								X			7									
Unidentified												X	X							
Coleoptera : Dytiscidae								5									X			
Buprestidae													X							
Coccinellidae																	X			
Unidentified larvae												2	4							
Unidentified terrestrial adults	1	1						X			7									
Trichoptera: Larvae and cases	4			43			75		13	5		7				40	23	33	8	53
Lepidoptera: Unidentified adults								3			5						X		X	4
Diptera : Chironomidae larvae and pupae	42	50	X	19				X	25	X	X	40	X		70	10	35	17	52	12
Simulidae larvae							X					1	X						X	4
Tipulidae larvae																	X		9	16
Ceratopogonidae larvae	X																		X	
Rhagonidae larvae	X																			
Asilidae adults																	X			
Unidentified larvae	2																X			
Unidentified adults		1						X			7									X
Hymenoptera: Formicidae			25	3				21		56	22	1	57							
Ichneumonidae								X												
Unidentified	3																6			
Unidentified insect remains	2		X	X								1				10				
Arachnida																				
Unidentified spider	X																			
Mollusca																				
Sphaeriidae	9			1						1		X	X		25		5			
Unidentified Pelecypoda													6	20			2			
Planorbis sp.																				1
Gyraulus sp.																				X
Lymnaea sp.	X							9	47	18		5	2							
Physa sp.																	2			
Egg mass	X																			
Mollusca																				
Unidentified Gastropoda	X	X		12		X				17					X		4			
Fish						33														
Gasterosteus aculeatus																				1
Oncorhynchus nerka fry																		50		
Unidentified																				
Algae																				
Nostoc sp.																	X		X	X
Filamentous forms																	X		1	2
Diatoms																	X			
Unidentified colonial forms																			X	
Miscellaneous																				
Remains of higher plants				2					X		X						X			
Seeds	X																			
Conifer needles																	X	X		
Organic debris				X			X		X										X	
Unidentified eggs									X	X										
Feathers																	X			
Wood	X	X		X			X					X					X	X		X
Sand, gravel, stones	X						X					X					X			X
Bottom ooze												X	X				X			
Unidentified material	1			4					2								X			
Total number of stomachs	35	20	3	14	1	3	2	14	17	22	3	51	19	2	3	1	42	3	26	38

and Plecoptera nymphs and Trichoptera larvae, are taken frequently, but there also occur a great variety of free-swimming and terrestrial or aerial organisms. These forms, which do not occur naturally on the bottom, may have been taken at any point between the bottom and the surface, or even while flying above the water.

Variation With Age

A comparison of tables I and II will illustrate differences in diet between yearlings and older fish. Size is probably the controlling factor in this regard, as demonstrated by the absence of the larger organisms from the stomachs of the younger individuals. As indicated in table III, the smaller organisms, such as larval Diptera (chiefly small Chironomidae larvae) and Entomostraca, form a large percentage of the food of younger fish, but occur in smaller proportions as the fish increase in age. Organisms of intermediate size, such as Ephemerida and Plecoptera nymphs, Trichoptera larvae and terrestrial insects are taken in greatest volumes by fish of intermediate age, while the largest forms, for example the Gastropoda, appear in largest quantities in the oldest individuals.

Seasonal Variation

Differences in food at various seasons of the year are illustrated by table IV. From January to March the stomachs contain aquatic insect larvae almost exclusively. In the period from April to June additional organisms begin to appear, as shown by the presence of Cladocera, fish, and terrestrial insects in the food. From July to September the variety of organisms taken is at its greatest. Aquatic insect material still appears in important quantities, but terrestrial insects, Cladocera, and many other seasonal forms are present in considerable numbers. The data presented by Munro and Clemens (1937) show a similar tendency to seasonal variation. Specimens with ripening gonads taken at Cultus lake in December had little or no food in the stomachs or intestines. This suggests that feeding is restricted to some extent towards spawning time. However, the lack of a complete series of samples throughout the winter makes it impossible to gauge accurately the extent of this period of fasting.

IMPORTANCE IN RELATION TO OTHER SPECIES

As stated previously, the Rocky Mountain whitefish is of some importance as a game and food fish. Dymond (1932) states that it will often rise to an artificial fly. Dill and Shapovalov (1939) agree with this author, pointing out that it puts up a good fight when hooked and that it is an excellent table fish. They list several reasons for the unpopularity of the species among anglers in California and point out that each is based on erroneous information. In lakes and rivers of the National Parks in Alberta this whitefish provides considerable fly and bait fishing, and regular "grayling" seasons occur when large numbers of anglers fish for the species. In British Columbia it is fished for food in many localities, and until a recent ban on its sale was ordered by the Department of Fisheries, it was sometimes peddled from door to door.

TABLE III. Percentage occurrence by volume of the principal food organisms in stomachs of *Prosopium williamsoni* at various ages.

Age (years)	I	II	III	IV	V	VI	VII	VIII	IX	X
Larval Diptera	62	39	31	32	12	20	14	22	13	10
Entomostraca	15	8	6	11	14		4		X	
Ephemerida and Plecoptera nymphs	15	30	9	11	15	15		2	1	1
Trichoptera larvae	1	8	11	10	26	39	15	14	8	10
Terrestrial insects	5	12	26	15	19	11	32	14	13	7
Oligochaeta			X	X		6	16		19	16
Pelecypoda			3	1	1	X	4	1	4	X
Gastropoda		2	3	2	X	5	13	15	33	39
Hirudinea						1			8	
Fish				4				14		17

TABLE IV. Percentage occurrence by volume of food organisms in stomachs of *Prosopium williamsoni* for each season of the year.

Months	Percentage volume of food organisms
January to March	Chironomidae larvae and pupae, 36; Trichoptera larvae, 26; Plecoptera nymphs, 15; Tipulidae larvae, 11; Ephemerida nymphs, 6; Neuroptera larvae, 1; Oligochaeta, X; Gastropoda, X; other aquatic insect larvae, X; Ostracoda, X; Algae, X; bottom debris, X.
April to June	Trichoptera larvae, 37; Chironomidae larvae and pupae, 29; Cladocera, 12; Gastropoda, 6; Pelecypoda, 5; Algae, 5; fish, 2; terrestrial insects, 1; Tipulidae larvae, 1; wood, X; bottom debris, X.
July to September	Chironomidae larvae and pupae, 27; terrestrial insects, 19; Gastropoda, 12; Trichoptera larvae, 8; Ephemerida nymphs, 7; Oligochaeta, 5; Cladocera, 5; Pelecypoda, 3; swimming insects, 3; other aquatic insect larvae, 2; Hirudinae, 1; Plecoptera nymphs, 1; Amphipoda, 1; unidentified insects, X; remains of higher plants, X; unidentified, X; Copepoda, X; fish, X; Mysis, X; spider, X; Ostracoda, X; Hydracarina, X; Algae, X; eggs, X; feathers, X; organic debris, X; wood, X; sand and gravel, X; mud, X.
December	In Cultus lake, little or no feeding.

On the other hand, the present investigation shows that the food of *Prosopium williamsoni* is very similar to that of trout and young salmon. Mottley and Mottley (1932) show that the Kamloops trout feeds to a considerable extent on aquatic insects and other bottom forms, while Foerster (1925) states that migrating sockeye salmon yearlings also feed largely on aquatic insect larvae. In highly productive waters these similarities would be of little consequence, but where the available food supply is limited competition might result.

In addition, it has been shown by the present data that this whitefish may destroy the young of other species to some extent, although Chapman and Quistorff (1938) do not believe it likely that *Prosopium* preys on the young

salmonoid fishes in the Columbia river. Furthermore, Foerster (1925) found sockeye salmon eggs in stomachs of this species, and is of the opinion that under certain conditions the Rocky Mountain whitefish might adversely affect the yield of a salmon river.

CONCLUSIONS

The Rocky Mountain whitefish is chiefly a bottom-feeding species, the most important food being aquatic insect larvae. The diet varies considerably with locality, and in waters which are poor in bottom fauna feeding may take place at any level, including the surface. The type of food taken changes as the fish increases in age, due to the selection of larger organisms by the larger fish. Variation in food with season depends on the abundance of the available organisms.

Although the species possesses qualities which make it desirable as a game and food fish, it also competes for food with, and at times takes the eggs and young of other species. For these reasons the desirability of its presence in any body of water must be considered in relation to the status of the other forms present. These interrelationships are rather complicated, and it is outside the scope of the present paper to make any definite statements in this regard.

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Periodicity in the Numbers of the Cladoceran *Scapholeberis mucronata*

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ABSTRACT

A periodicity of 21 to 28 days in the numbers of *Scapholeberis mucronata* (O. F. Mueller) was disclosed by weekly sampling of four separate populations during two years. The causes of the periodicity were not clearly defined, although certain correlations between the numbers of *Scapholeberis* and fluctuations of the algal populations and chemical factors of the medium were suggestive.

Periodic fluctuations in the growth and numbers of plants and animals have, in certain instances, been on record for years. However, only in comparatively recent times have studies been undertaken to ascertain more accurately the nature and scope of these fluctuations.

In this paper attention is drawn to a periodicity of short duration in the numbers of the cladoceran, *Scapholeberis mucronata* (O. F. Mueller).

HABITATS

The following observations were made in three experimental ponds at the Atlantic Biological Station, St. Andrews, New Brunswick. Two of the ponds are oval in shape, with sloping sides, of cement structure, and hold approximately 35 cubic metres of water. The third pond is round, with sloping sides of cement structure lined with brick, and has a capacity of approximately 33 cubic metres. Hereafter the former ponds will be designated as 1 and 3, and the latter pond as 2.

When the observations were made, the ponds were being used in experiments designed to illustrate the effect of an organic fertilizer upon the production of fresh-water plankton. The first observations were made in 1930 in ponds 1 and 3. Pond 1 received a fertilizing of 0.013 gram of herring meal per litre of water, while pond 3 served as a control and was not fertilized. The other observations were made in 1933, at which time ponds 1 and 2 were involved. Both received 0.05 gram of herring meal per litre of water, but applied in two ways, in gunny sacks in pond 1 and loosely in pond 2.

METHODS

The samples of *Scapholeberis* were secured weekly, between 10.00 a.m. and 12.00 noon, by dipping and straining 10 litres of water through a number 18 bolting silk plankton net. The counts were made by enumerating the organisms in 10 to 50 per cent of each sample, depending upon the number. Samples were taken from three levels: surface (0-10 centimetres), half-depth and bottom. *Scapholeberis* is characteristically a surface-haunting form, with the result that these cladocerans were largely confined to the upper 10 centimetres of the waters. On most occasions the number secured in either the half-depth or bottom samples was less than 5 per cent of that taken at the surface. On other infrequent occasions this percentage was somewhat higher. An extreme case was that of July 25, 1933, in pond 2, when the number at half-depth was 21 per cent and at bottom 10 per cent of the number secured in the surface sample. However, only surface samples are herein considered, for upon analysis it was found that the numbers at the lower levels did not alter the trend of the populations as illustrated in figures 1, 2, 3, and 4.

The dissolved oxygen content of the water was determined by the Winkler method and the carbonate content (phenolphthalein alkalinity) by titration with 0.02 N sulphuric acid. The pH values were secured colorimetrically with thymol blue and phenolphthalein.

OBSERVATIONS

An examination of figures 1A, 2A, 3A and 4A shows that maxima in the numbers of *Scapholeberis* reappeared in a periodic manner at intervals from three to four weeks. The population in pond 1 exhibited during 1930 three points of maximal production—on July 8 and 29 and on August 19 (fig. 1A). Between August 19 and September 16 the number of *Scapholeberis* in this pond gradually decreased, then declined more suddenly between the latter date and September 23. Thus, in addition to the points of maximal production mentioned above there was a point of fairly abrupt change in numbers of the cladoceran on September 16. A similar consideration of figure 2A for the production in pond 3 during 1930 also shows maxima on July 8 and 29 and on August 19. From the observations made in 1933, maximal numbers of *Scapholeberis* were found in pond 1 on July 24, August 21 and September 11 (fig. 3A), and in pond 2 on July 17, August 7 and 28 (fig. 4A). In contrast with the cases of periodicity noted in 1930, the points of greatest abundance were not simultaneous in the two ponds in 1933. The period, however, was the same, namely three to four weeks.

Since the samples were collected weekly, the number of days for the cycle in the numbers of *Scapholeberis* cannot be precisely defined. As indicated by the data, however, the period was between 21 and 28 days, and approximated more closely 21 days.

Many limnologists have reported upon the seasonal pulses in the number of cladocerans, and other entomostracans, in ponds and lakes. These pulses are closely associated with changes in the various physical and biological conditions within the habitats as they recur with each particular season, and accordingly

their appearance may vary in point of time from year to year. These long-term, and often erratic, fluctuations are not considered comparable to the short-period cycle in the numbers of *Scapholeberis*. More comparable with the observations on *Scapholeberis* are those of Weigold (1910). He reported maxima in the num-

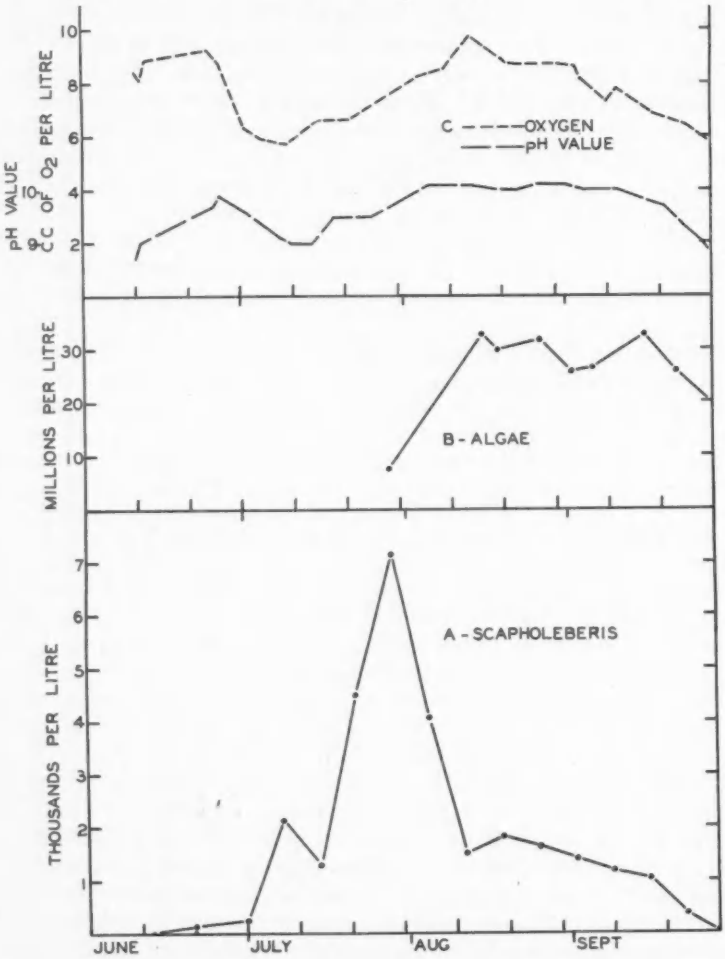

FIGURE 1. Numbers of *Scapholeberis* and algae, and values of the dissolved oxygen content and pH of the water, pond 1, 1930.

bers of *Chydorus sphaericus* in certain Saxe waters occurring in April (the largest), June, August and November (the smallest), but the reasons for this phenomenon, he stated, were not clear. In criticism of Weigold's work Berg and Nygaard (1929, p. 251) remarked: "It may be doubtful whether more precise methods

than those employed by Weigold would readily confirm a periodicity of this description." Other references to Cladocera, similar to Weigold's observations, appear lacking in the literature.

Cycles in the abundance of plants and animals are not uncommon, such as those for insects, fish, birds, bacteria and animal parasites that were reported to the Matamek conference on biological cycles (Huntington 1932; Elton 1933).

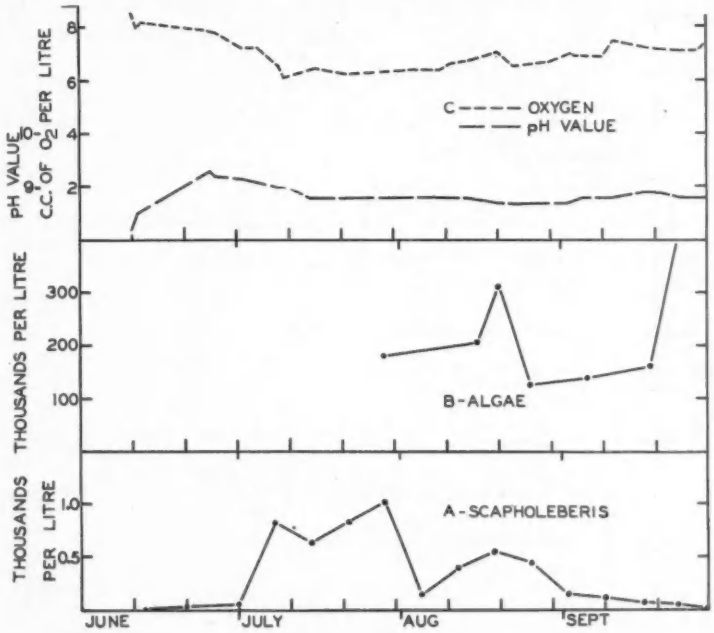

FIGURE 2. Numbers of *Scapholeberis* and algae, and values of the dissolved oxygen content and pH of the water, pond 3, 1930.

RELATION TO ALGAL POPULATIONS

Algae, either as living cells or as a source of decomposing organic matter, constitute a major food supply for cladocerans in natural habitats. The dependence of *Scapholeberis* upon algae as food is well illustrated in figures 3 and 4. The fertilizer was added loosely to the water in pond 2 in 1933, but was confined in sacks in pond 1. As a result, the growth of algae was stimulated more quickly in the former pond than in the latter. Sharp increases in the numbers of *Scapholeberis* followed the algal pulses in both ponds. However, due to the difference in time of the algal pulses, the first cladoceran maximum was reached in pond 2 on July 17, while that in pond 1 was not realized until July 24. To all appearances this initial relationship between *Scapholeberis* and algae accounted for the asynchronism between the minima and maxima of the cladoceran production in the two ponds (cf. figs. 3A and 4A).

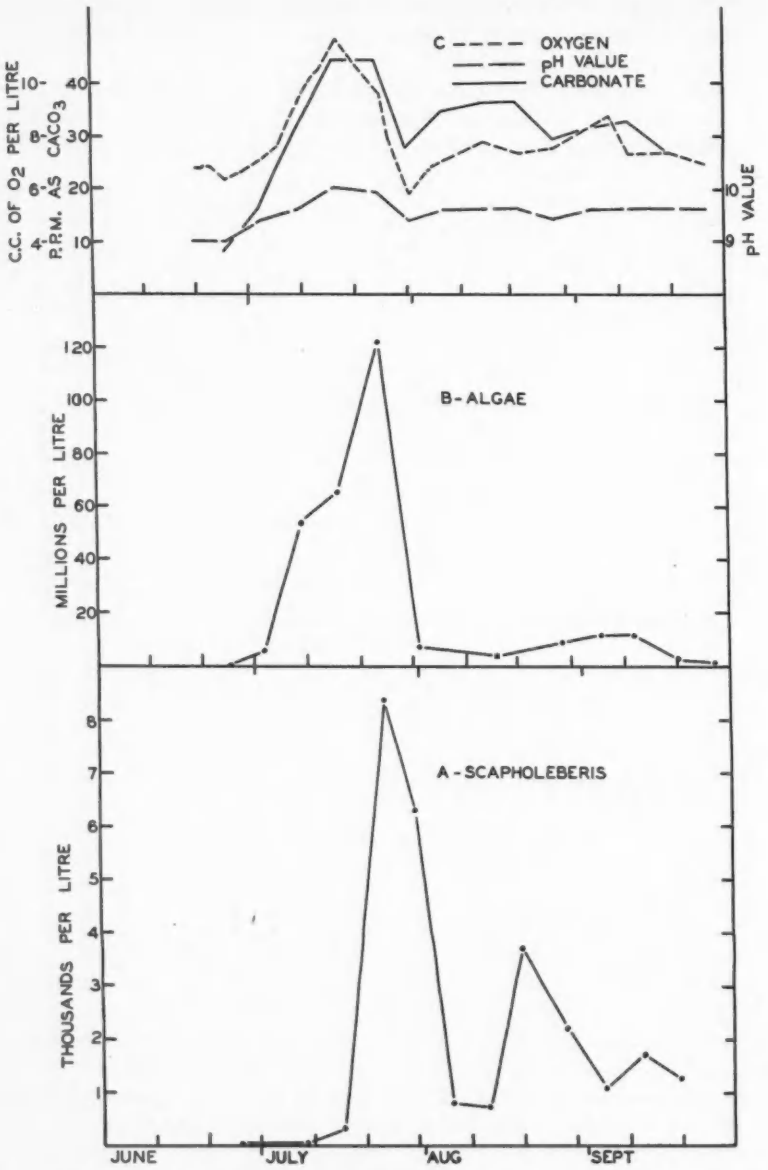

FIGURE 3. Numbers of *Scapholeberis* and algae, and values of the dissolved oxygen and carbonate content and pH of the water, pond 1, 1933.

In 1930 algal samples were not secured until after *Scapholeberis* was well established in the ponds. Nevertheless, the oxygen and pH records indicate that a considerable algal population had been present in the ponds a short time prior to the first *Scapholeberis* maxima (figs. 1C and 2C).

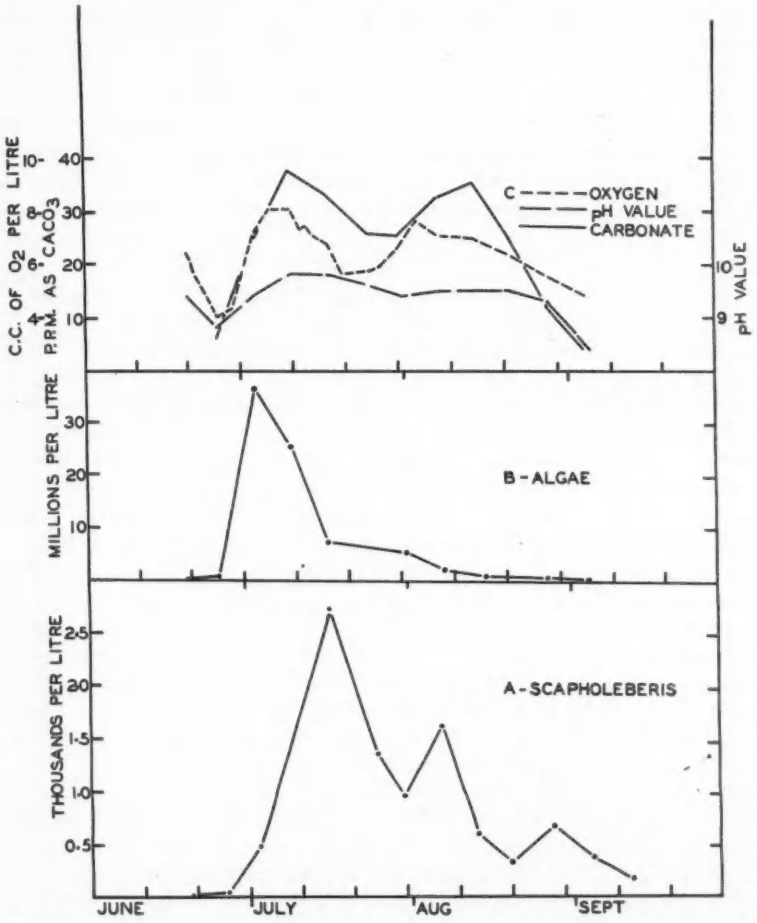

FIGURE 4. Numbers of *Scapholeberis* and algae, and values of the dissolved oxygen and carbonate content and pH of the water, pond 2, 1933.

As the season progressed in 1933 and the algae became scarcer, the *Scapholeberis* populations declined—the peaks of maximal numbers became lower (figs. 3A and 4A). In 1930, on the other hand, the *Scapholeberis* populations decreased while the algae were still plentiful (figs. 1 and 2). Thus, although the food relationship between *Scapholeberis* and algae was important, yet other factors must

have also influenced the maintenance and decrease of cladoceran populations as a whole.

Since algae, as a food supply, had undoubtedly much influence upon the numbers of *Scapholeberis*, any periodic fluctuations in the abundance of algae would probably be reflected by the cladocerans. As we have seen, the rise to and fall from the initial maxima in the numbers of *Scapholeberis* were correlated with peaks in the algal growth, particularly in ponds 1 and 2 in 1933 (figs. 3 and 4). Again in mid-September, 1933, an increase in the numbers of *Scapholeberis* in pond 1 was correlated with an increase in the numbers of algae (fig. 3). Further, as shown in figure 2, the cladoceran maximum in August was coincident with a larger algal production. Beyond these cases, however, there was little direct evidence that the periodicity in the numbers of *Scapholeberis* was correlated with fluctuations in the algal populations, and thus possibly determined by them.

RELATION TO CHEMICAL FACTORS

The fluctuations in dissolved oxygen and carbonate content and pH value of the water were dependent upon the variations in the abundance of algae. The relationship was not only dependent directly upon the algae through photosynthesis and respiration, but less directly through the decomposition of algal cells. Respiration of the zooplankters also had an effect, although it is considered to have been of comparatively minor importance. At certain times the photosynthetic activities of the algae dominated, and the values of oxygen, carbonate and pH were high. At other periods the utilization of oxygen and liberation of carbon dioxide by decomposition depressed these values. A comparison of figures 3B and 3C illustrates these interrelations. A point of interest shown by the diagrams is that oxygen, carbonate and pH values rose while the algal population was comparatively small in August. Apparently the photosynthetic action of the smaller, yet appreciable, algal population was sufficient to cause a recovery in the values of the chemical factors as soon as the decomposition effects, accompanying the sudden decline in the numbers of algae in late July, had passed. The values of the chemical factors decreased, however, as the algal population reached a low ebb in mid-August, to recover again, but at a lower level, when the small algal pulse developed in early September.

In figures 1 and 4 the relationship between algae and the chemical factors is also shown, but in figure 2 this is less clear, probably due to the small number of algae present in the unfertilized water of pond 3 (1930).

In figure 3 a correlation between the *Scapholeberis* maxima and the higher values of dissolved oxygen, carbonate and pH is illustrated. Similarly in figure 4 the first two maxima are likewise correlated. The correlation was not manifest throughout all the data, however, and, as with the algae, the evidence at the most was only partial that the periodicity in the numbers of *Scapholeberis* was connected with the variations in oxygen and carbonate content and pH value of the water. These variations in the chemical conditions were determined largely by the algae, either directly by photosynthetic activity or indirectly by their decomposition, and were, therefore, secondary in time of occurrence, although they may have been primary in function.

RELATION TO TEMPERATURE AND METEOROLOGICAL CONDITIONS

The surface temperature of the water in pond 1 for morning (9.00 to 10.00 a.m.) and afternoon (4.00 to 5.00 p.m.) are given in figure 5 for 1930 and 1933. The temperature of the water in ponds 2 and 3 agreed closely to that found in pond 1 for the two years under consideration, and accordingly the records for these ponds are not presented. The seasonal trend of the water temperatures exhibited higher and lower levels but in no definite cyclic manner. There was thus no obvious correlation between the temperature of the water and the cladoceran cycle.

As far as these investigations went, meteorological conditions had no relation to the periodicity. Records were kept for morning and afternoon of the local air temperature, sky conditions and wind direction and velocity. No periodicity was evident in the trend of these climatic factors. The nature of the cladoceran

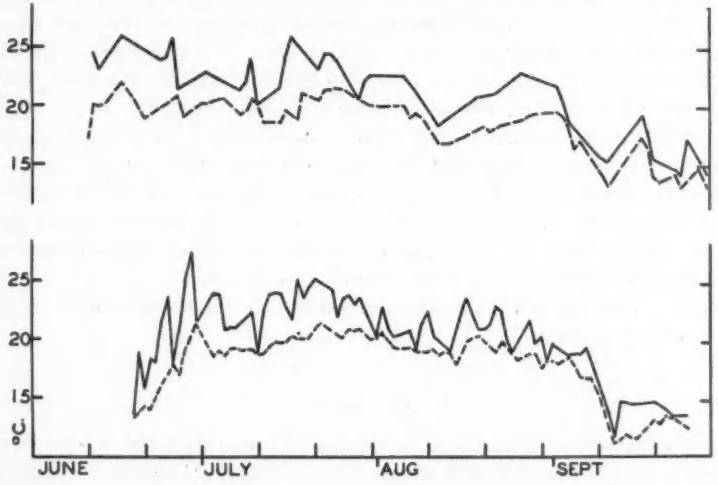

FIGURE 5. Temperatures of the surface water, pond 1. Above for 1930 and below for 1933. ------ a.m., ——— p.m.

production in ponds 1 and 2 during 1933 appeared to exclude extra-environmental forces as determining influences. Although the cycle covered the same interval of time in both ponds, i.e. as close as could be indicated by the method of sampling, a maximum occurred in one while a minimum was being experienced in the other (cf. figs. 3A and 4A). Meteorological factors, if they had operated to condition the cyclic character of the *Scapholeberis* production, would have tended to disrupt the periodic appearance of minima and maxima in one or other of the ponds and to make the period synchronous in both ponds.

RELATION TO SEXUAL REPRODUCTION

Sexual reproduction was encountered in the cultures of *Scapholeberis*, and when it did occur it proved periodic in character. The greatest numbers of females bearing ephippia were taken either when the numbers of the cladocerans

were at a maximum, or as they were declining. Ephippial females were absent, or present in very small numbers, as the production was at a minimum or was on the upward trend. On certain similar occasions males were also present with the ephippial females, although the data upon the occurrence of males are less complete.

Weismann (1879) contended that the onset of sexual reproduction in Cladocera was independent of the environmental conditions, and was determined by an innate sexual rhythm peculiar to such species. The Weismannian contention was rejected and disproved by Agar (1914), Banta (1914, 1915, 1925), Berg (1931) and others who were able to carry Cladocera through many parthenogenetically-produced generations without the appearance of gamogenesis. The view generally accepted by most of the recent investigators is that sexuality in Cladocera is determined by unfavourable changes in the environmental factors. Much work, well reviewed by Tauson (1930) and Berg (1931), has been published, purporting to demonstrate the causal relationship between one or more of the environmental conditions and the onset of sexuality. Among those factors for which the causal effect has been best demonstrated are temperature (Issakowitsch 1905; Papanicolau 1910; Tauson 1930; Banta and Brown 1925), reaction of the medium (Tauson 1930), food (Kerhevé 1892, 1895; Tauson 1930), and possibly crowding and excretory products (Berg 1931; Banta and Brown 1929a, 1929b).

Since the appearance of sexual forms among *Scapholeberis* was periodic, the evidence advanced in the literature that environmental factors initiate gamogenesis is corroborative of the suggestions made above that fluctuations in the algal populations and in the chemical conditions of the water, as controlled by algal growth, were responsible, in part at least, for the observed periodicity in the numbers of *Scapholeberis*.

CONCLUSIONS

The periodicity in the numbers of *Scapholeberis* was observed in four separate populations during two different years. It is concluded, therefore, that these observations were not coincidental, but illustrate a natural phenomenon. In view of the fact that periodicity in the numbers of various species of animals is of widespread occurrence, we may speculate that it is a more common event among populations of Cladocera, and other entomostracans, than now appreciated, and that it requires regular sampling, particularly when the animals are abundant, to bring the phenomenon to light.

The data present no precise evidence of the cause of the periodicity in the numbers of *Scapholeberis*. Correlations do suggest, however, that algae (as food) and chemical factors of the medium, as influenced by the growth of algae, were important in this regard. Many conditions in the habitats were not investigated, so it is not surprising that the cause of the periodicity was not more definitely established.

From the data at hand, the conclusion is drawn provisionally that the periodicity was determined by internal environmental factors of the habitats, rather than by outside influences or any predisposition to a cyclic production inherent in *Scapholeberis* itself.

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The Comparative Value of Preservatives for Fresh Fillets

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ABSTRACT

The effect of a number of chemical preservatives in inhibiting the bacterial "spoilage" of fresh fillets of certain species of Pacific coast fish has been studied. The preservatives were applied by dissolving them in sodium chloride brines in which the fish were immersed. Chloroform (0.7%) markedly improved the keeping quality of treated fish, while hydrogen peroxide (0.1%) had little effect. Sulphurous acid (0.1% SO_2) considerably retarded bacterial "spoilage" but produced an unpleasant flavour. Hydrochloric acid (0.1%) slightly enhanced keeping quality but caused treated fillets to assume an unattractive appearance. Benzoic acid (0.1%) and sodium benzoate (0.1%) were quite effective, the former being slightly more active than the latter. Neither boric acid (0.1%) nor para-hydroxybenzoic acid ethyl ester (0.09%) were as efficient preservatives as benzoic acid or sodium benzoate in similar (per cent) concentration. Both sodium nitrite and potassium nitrite in 0.1% concentration caused a much greater inhibition in bacterial "spoilage" of fresh fillets than did sodium benzoate or benzoic acid, and the probable value of nitrites in fish preservation, as well as their influence on the colour of treated products, is discussed. The efficiency of a given preservative in different cases varied greatly, and in certain experiments a compound which normally exerted a favourable effect on keeping quality was without effect. The probable reason for this is discussed.

Most attempts to improve the keeping quality of dressed fish by immersing them in solutions containing bactericidal substances, or by surrounding them with ice in which germicidal compounds have been incorporated, have resulted in failure, or only in a small measure of success, as has previously been pointed out (Tarr and Sunderland 1939a; Tarr and Bailey 1939). The reason for such failures undoubtedly lies in the fact that it is extremely difficult to ensure intimate contact between putrefactive agent and chemical preservative in experiments with dressed fish. One method which appeared to offer considerable possibilities, and which undoubtedly greatly enhances the keeping quality of treated fish, namely that of immersing them in strong salt solutions on board ship during the interval between dressing and icing them (Bedford 1932), has never been adopted. Such treatment so alters their external appearance as to make them unacceptable according to the grading methods in vogue. However, in excised fish muscle (fillets, steaks, etc.) contact between preservative and bacteria is facilitated, and recent work has been directed largely to the study of such products.

Since about 1870 numerous publications have appeared regarding the preservation of fish and fish products by chemical means, and only brief reference can be made to them here. At one time boric acid was advocated as a preservative, and was used to prevent reddening of salted fish (Ewart 1886; Stevenson 1899; Tressler 1923; Bronkhorst 1926; Cobb 1927), but more recently benzoates have been employed for these purposes (Cobb 1927; Gibbons 1935). In recent years such varied agents as acids (Metzner 1933b; Gibbons 1934; Notevarp, Hjorth-Hansen and Monssen 1936; Nadeau 1939), carbon dioxide (Killeffer 1930; Coyne 1933; Stansby and Griffiths 1935), ultraviolet light (Tarr, Young and Sunderland 1938; Puncochar, Lanham and Nilson 1939) and hydrogen peroxide (Metzner and Oeser 1938; Metzner, Hutschenreuter and Oeser 1938; Lücke 1938; and Hjorth-Hansen and Karlsen 1939) have been employed to preserve fish.

The literature pertaining to the use of germicidal substances in ices has already been reviewed (Tarr and Bailey 1939), and brief reference has also been made to experiments dealing with the preservation of lightly smoked and fresh fillets (Tarr and Sunderland 1938, 1939a and b). A detailed report of the experiments on the preservation of fresh fillets follows.

METHODS

Bacteria produce changes in fish which are more or less objectionable to those who prefer really fresh fish, and such changes may be called "spoilage."

No single and entirely satisfactory chemical, physical or biological test by which the degree of bacterial "spoilage" of all kinds of sea fish can be accurately and quantitatively measured, has yet been found. For the purpose of the experiments to be described viable bacterial counts and organoleptic tests were arbitrarily selected as the best available criteria of bacterial "spoilage". The "trimethylamine test," which has apparently given quite satisfactory results under certain conditions, was used only in one experiment, since benzoic acid, benzoates and para-hydroxybenzoic acid ethyl ester markedly suppress trimethylamine formation in "spoiling" fish muscle without effecting a corresponding inhibition in bacterial multiplication, as this and previous work (Tarr and Sunderland 1938; Tarr and Bailey 1939) has shown. Also, only certain of the organisms causing fish "spoilage" form trimethylamine (Watson 1939; Tarr 1938, 1939).

Viable bacterial counts and trimethylamine determinations were made by methods previously described (Tarr and Bailey 1939). The amount of sodium nitrite in treated fish was determined as follows. Aqueous extracts of the muscle were prepared following the technique used in making bacterial counts, the protein was precipitated with mercuric chloride solution, and the amount of nitrite present in the filtrate, or in suitable dilutions prepared from it, was determined colorimetrically, employing the Greiss-Ilsovay reagent in the method outlined by the Association of Official Agricultural Chemists (1925).

Organoleptic tests were made by the writers, though, whenever possible, the assistance of others was obtained. These tests included examination of the uncooked fish for odour, and of the fish baked for 10 minutes at about 300°C.

in an electric oven for both odour and flavour. For purposes of brevity the results of organoleptic tests have been recorded as in table I. Organoleptic tests have been made only in experiments where it was considered that they would give information of value.

In the first few experiments small fillets which were cut to approximately the same length, width and thickness were used. Later this technique was altered, pieces of fish muscle cut to standard size being treated, thereby ensuring a more uniform penetration of preservative or sodium chloride. In all experiments the skin was removed from the fillets. Tests were at first carried out by dipping the fillets in 2 per cent sodium chloride brines at a temperature of approximately 20°C., with or without added preservative. This technique was subsequently altered when it became evident that a brief immersion of fillets in strong sodium chloride brines was itself a strong deterrent to bacterial putrefaction, and that, for reasons to be given later, brines kept at temperatures near the freezing point of water were preferable in certain respects to those used at higher temperatures. After the brine treatment the fillets were usually placed in sterilized 500- or 750-ml. covered glass beakers or 20-cm. petri dishes, though in certain experiments they were wrapped in cellophane paper. The fish was usually stored at 1.5°C., though occasionally a higher temperature was employed. The liquid which accumulated was drained from the containers daily. In most experiments the samples were stored until the untreated fish became stale, when all fillets were examined. Lower bacterial counts and better odour and flavour in treated than in untreated samples were taken to indicate an improvement in keeping quality. In other experiments a comparison of the length of time required for treated and untreated fillets to arrive at approximately the same stage of staleness, as indicated by viable bacterial counts and odour, was used as a criterion of improvement in keeping quality.

Usually it has been possible to give only a very approximate estimate of the number of days which elapsed between the catching of the fish and the time at which they were used for experimental purposes. Thus the expression, "five days old in ice" signifies that the fish used were stored for about five days in ice during the time which elapsed between capture (dressing and washing) and experimental use.

EXPERIMENTAL

A. Fillets Treated in Weak (2%) Sodium Chloride Brines

EXPERIMENT 1.

One-litre portions each of five solutions having the composition shown in table I were prepared. Small flounders about 1 day old in ice were filleted and 200 ± 10 g. of the fillets were immersed in each of these solutions for 1 hour at about 20°C. with occasional stirring. After 3 days' storage at 1.5°C., 3 fillets were removed from each container, and, after mincing them together with sterile scissors in the usual manner, viable bacterial counts and determinations of the trimethylamine content of the fish were made, with the results given in table I The remaining fillets were re-incubated at 22°C. for 17 hours in order to accelerate decomposition, after which further examinations were made (table I).

TABLE I. Experiment 1. Spoilage (as judged by numbers of viable bacteria, trimethylamine content and organoleptic tests) of flounder fillets after treating in 2% sodium chloride brine with the preservatives listed.

Preservative	Stored 3 days at 1.5°C.		Stored 3 days at 1.5°C. +17 hr. at 22°C.		Order of preference of treatment according to organoleptic tests**	
	Bacterial counts*	Mg. trimethylamine nitrogen per 100 g.	Bacterial counts*	Mg. trimethylamine nitrogen per 100 g.	Fish uncooked (3 judges)	Fish cooked (2 judges)
1. None	20,200	0.17	26.4×10^6	24.2	4, 4, 3	2, 3
2. 0.1% benzoic acid	4,760	0.18	0.8×10^6	0.32	1, 1, 1	1, 1
3. 0.09%para-hydroxybenzoic acid ethyl ester	4,920	0.14	8.7×10^6	0.37	1, 1, 1	1, 1
4. 0.1% sulphur dioxide	5,800	1.03	0.25×10^6	13.2	5, 5, 5	3, 5
5. 0.1% hydrogen peroxide	9,920	0.13	11.5×10^6	10.6	3, 3, 4	2, 4

*In this and in subsequent tables bacterial counts are given as numbers of colonies per g. of wet fish muscle, the figures representing averages of duplicate determinations.

**In cases where there was equivalence of preferences the samples in question were given the same number. Thus in the above table the order of preference according to cooked flavour was in one case (2, 3) (1, 5), 4 (brackets indicating equivalence of preference). This was therefore recorded as first place for samples 2 and 3; second place for samples 1 and 5 and third place for sample 4.

It is evident that all of the four preservatives studied retarded to a greater or lesser degree the bacterial "spoilage" of flounder muscle. The effect of the preservatives was more marked after the longer storage period in the case of the benzoic acid and sulphurous acid treatments, as judged by bacterial counts; but this is not true for hydrogen peroxide, a result which might be expected since this compound has a purely transient effect, being rapidly decomposed, presumably by the catalase present in the muscle. Both benzoic acid and para-hydroxybenzoic acid ethyl ester gave considerable protective action, though the former was more effective than the latter. Both almost entirely suppressed trimethylamine formation in the treated muscle without effecting nearly such a marked inhibition in the increase in viable bacteria. Sulphur dioxide had a strong bactericidal action, but produced such an unpleasant rancid odour and flavour in the treated fish, presumably due to its reaction with the oils present (Denstedt and Brocklesby 1935) that its use was not considered further.

Sulphur dioxide apparently effects a chemical reduction of trimethylamine oxide to trimethylamine, for the increase in the viable bacterial population is hardly great enough to account for the relatively large amount of trimethylamine in the treated muscle.

EXPERIMENT 2

The relative effectiveness of benzoic acid and sodium benzoate as preservatives for fresh fillets was determined, the technique being similar to that followed in experiment 1. Small flounders 3 days old in ice were filleted and 12 fillets (150 to 160 g. of fish) were immersed for 1 hour at about 20°C. in 1-litre portions of each of 5 brines (table II). The treated fillets were stored at 1.5°C. for 10 days. Viable bacterial counts were then made after mincing together 3 fillets selected at random from each of the 5 lots, and also organoleptic tests on the remaining fillets (table II). Both bacterial counts and organoleptic tests indicate a marked superiority in keeping quality of the treated over the untreated fillets. The viable bacterial counts showed that 0.1% benzoic acid was only slightly superior as a preservative to 0.1% sodium benzoate, the difference obtained being hardly significant. This result is probably explained by the fact that, although the free acid is a much more effective germicide than the benzoate (Goshorn, Degering and Tetrault 1938), it is possibly neutralized as soon as it comes in contact with the fish muscle. It will be noticed that the organoleptic tests were not sufficiently sensitive to indicate significant differences among the fillets treated with preservatives.

TABLE II. Experiment 2. "Spoilage" (as judged by numbers of viable bacteria and organoleptic tests) of flounder fillets after treating in 2% sodium chloride brine with the preservatives listed.

Preservative	Bacterial counts	Order of preference of treatment according to organoleptic tests tests (3 judges)	
		Fish uncooked	Fish cooked
1. None	1398 $\times 10^6$	5, 5, 2	5, 5, 3
2. 0.1% benzoic acid	46.6 $\times 10^6$	1, 1, 4	1, 1, 3
3. 0.1% sodium benzoate	144.8 $\times 10^6$	2, 3, 4	2, 4, 4
4. 0.5% sodium benzoate	3.3 $\times 10^6$	2, 2, 2	2, 2, 2
5. 1.0% sodium benzoate	0.19 $\times 10^6$	1, 2, 3	1, 3, 2

EXPERIMENT 3

A halibut (*Hippoglossus stenolepis*) weighing about 6 lb. (3 kg.) which had been air frozen when still fresh, wrapped in cellophane and stored 5 months at −20°C., was thawed by placing in a 20°C. incubator for 16 hours. Pieces of muscle approximately 7.5×7.5×2.0 cm. were cut from this fish, and 4 of these brined for 1 hour at approximately 20°C. in 1-litre portions of each of the 4 solutions, the composition of which is given in table III. After 9 days' storage at 1.5°C., 2 pieces of muscle from each lot were minced together for bacterial counts, the remaining 2 being used for organoleptic tests (table III).

In this experiment it will be observed that the differences obtained between treated and untreated fish were insignificant as far as bacterial counts were concerned, while the results of organoleptic tests were not very conclusive in that

Table III. Experiment 3. "Spoilage" (as judged by numbers of viable bacteria and organoleptic tests) of halibut fillets in 2% sodium chloride brine after treating with the preservatives listed.

Preservative	Bacterial counts	Order of preference of treatment according to organoleptic tests (3 judges)	
		Fish uncooked	Fish cooked
1. None	1146×10^6	3, 4, 4	2, 3, 4
2. 0.1% benzoic acid	1034×10^6	1, 2, 3	2, 2, 2
3. 0.09% para-hydroxybenzoic acid ethyl ester	812×10^6	1, 1, 1	1, 1, 1
4. 0.1% sodium benzoate	1610×10^6	2, 2, 3	3, 3, 4

they did not in all cases indicate preference for the treated samples. The probable reason for this apparent discrepancy is discussed on page 160.

EXPERIMENT 4

Small fillets were cut from flounders which were less than 1 day old in ice, and 6 were immersed for 1 hour at about 20°C. in 1-litre portions of each of 6 brines, the composition of which is given in table IV. The fillets were stored at

TABLE IV. Experiment 4. "Spoilage" (as judged by numbers of viable bacteria and organoleptic tests) of flounder fillets after treating in 2% sodium chloride brines with the preservatives listed.

Preservatives	Wt. of fish added (g.)	Bacterial counts	Order of preference of treatment according to organoleptic tests (3 judges)	
			Fish uncooked	Fish cooked
1. None	167	80.0×10^6	3, 5, 5	2, 3, 4
2. 0.7% chloroform (approx.)	165	0.26×10^6	1, 2, 3	2, 2, 3
3. 0.1% potassium nitrite	180	0.48×10^6	1, 4, 1	1, 2, 5
4. 0.1% boric acid	180	8.2×10^6	1, 2, 3	1, 2, 6
5. 0.073% hydrochloric acid	170	1.5×10^6	2, 5, 5	1, 1, 3
6. 0.1% benzoic acid	181	14.4×10^6	1, 1, 4	2, 2, 3

1.5°C. for 10 days in the usual manner, and at the end of this time 2 from each lot were subjected to bacteriological analysis, the remainder being used for organoleptic tests (table IV). The results of the bacteriological tests show that both potassium nitrite and chloroform treatment caused an extremely marked improvement in keeping quality. Hydrochloric acid, boric acid and benzoic acid, while definitely retarding bacterial "spoilage," did not produce such a noticeable improvement as the first named compounds. Organoleptic tests did not indicate

such marked differences as the viable bacterial counts, especially when the cooked fish was employed as criterion. It appears that bacteriological decomposition had not proceeded far enough to cause obvious differences in the flavour of the cooked samples; there is no doubt that many of the volatile compounds which aid in detecting differences by smell alone are driven off during the cooking process.

EXPERIMENT 5

An air-frozen halibut weighing about 10 lb. (4.5 kg.), which had been frozen when fresh and stored for 5 to 6 months at −20°C., was thawed at 22°C. Sixteen pieces of the muscle about 7.5×5×1.5 to 2.0 cm. thick were cut, and 4 of these treated for 1 hour at approximately 20°C. in 1-litre portions of each of the 4 solutions, the composition of which is given in table V. After storing 12 days at 1.5°C., 2 fillets from each lot were used for determination of numbers

TABLE V. Experiment 5. "Spoilage" (as judged by numbers of viable bacteria and organoleptic tests) of halibut fillets after treating with various concentrations of potassium nitrite in 2% sodium chloride brine.

Percentage of potassium nitrite	Wt. of fish added (g.)	Bacterial counts	Order of preference of treatment according to organoleptic tests (3 judges)	
			Fish uncooked	Fish cooked
1. None	250	964 $\times 10^6$	2, 4, 4	4, 4, 4
2. 0.02%	274	158 $\times 10^6$	1, 2, 3	3, 3, 3
3. 0.05%	263	20.6 $\times 10^6$	1, 1, 3	1, 2, 2
4. 0.10%	254	0.66 $\times 10^6$	1, 1, 2	1, 1, 2

of viable bacteria, and 2 for organoleptic tests (table V). The results show that potassium nitrite treatment very markedly improves the keeping quality of halibut fillets both as regards bacterial counts and organoleptic tests.

At this stage it was realized that more uniform results as regards penetration of preservative and salt could be expected by using pieces of fish muscle cut to a definite size, and this was done for all subsequent experiments.

EXPERIMENT 6

Red cod (genus *Sebastodes*) about 2 days old in ice were filleted and 36 pieces of muscle 5×5×2 cm. were cut from them. Nine different brines in quantities of 1 litre were prepared (table VI), and 4 pieces of red cod were immersed in each of them for 1 hour at about 20°C. After 12 days' storage at 1.5°C. the fillets were examined in the usual manner. In this experiment viable bacterial counts (table VI) showed no significant differences in keeping quality of the treated fillets, with the single exception of those exposed to chloroform, as compared with those not treated with preservatives. Organoleptic tests indi-

TABLE VI. Experiment 6. "Spoilage" (as judged by viable bacterial counts) of red cod fillets after treating in 2% sodium chloride brine with the preservatives listed.

Preservative	Bacterial counts
None	296×10^6
0.05% sodium nitrite	270×10^6
0.05% potassium nitrite	284×10^6
0.10% sodium benzoate	1686×10^6
0.10% benzoic acid	1360×10^6
0.10% hydrogen peroxide	1104×10^6
0.09% para-hydroxybenzoic acid ethyl ester	1152×10^6
0.7% chloroform	0.114×10^6
0.1% boric acid	342×10^6

cated that only in the case of the chloroform treated fish was there any noticeable improvement in the condition of the fillets. Almost identical results were obtained when using halibut fillets in another similar experiment with the same preservatives.

EXPERIMENT 7

Sixteen pieces of muscle 5×5×2 cm. were cut from a halibut about 7 days old in ice. One-litre quantities of 4 brines, the composition of which is recorded in table VII, were prepared, and 4 pieces of the fish were immersed in each

TABLE VII. Experiment 7. "Spoilage" (as judged by viable bacterial counts and organoleptic tests) and sodium nitrite content of halibut fillets after treating with various concentrations of this preservative in 2% sodium chloride brine.

Percentage of sodium nitrite	Sodium nitrite in p.p.m. of wet halibut muscle after storing at at 1.5°C. for:		Bacterial counts	Order of preference of treatment according to organoleptic tests (2 judges)	
	3 days	6 days		Fish uncooked	Fish cooked
1. None	Not appreciable	Not appreciable	$1{,}906\times10^6$	4, 4	4, 4
2. 0.02%	42	9	$1{,}626\times10^6$	3, 3	3, 3
3. 0.05%	124	27	852×10^6	2, 2	2, 2
4. 0.10%	184	72	4.5×10^6	1, 1	1, 1

of these for 1 hour at a temperature of about 20°C. The amount of sodium nitrite present in the treated muscle was determined after 3 and 6 days' storage at 1.5°C., 1 fillet being used for the determination after the 3-day period (table VII). The number of viable bacteria in 1 fillet from each lot was determined after 6 days at 1.5°C., organoleptic tests being made on the remaining fillets (table VII). The results show that sodium nitrite, in concentrations lower than 200 parts per million (p.p.m.), strongly retarded bacteriological "spoilage" in the treated fillets. It will also be observed that the amount of nitrite in the

treated fish decreases on storage, presumably due to its reduction to ammonia by certain of the bacteria present.

EXPERIMENT 8

Four pieces of halibut muscle 5×5×2.5 cm. cut from a fish 5 days old in ice were immersed for 5 hours in 1-litre portions of 2 different brines (table VIII) which were maintained at 7 to 8°C. Sodium nitrite was determined in one fillet from each brine immediately subsequent to brining, and again after 9 days' storage of the remaining fish at 1.5°C., when bacteriological counts and organo-

TABLE VIII. Experiment 8. "Spoilage" (as judged by viable bacterial counts) and sodium nitrite content of halibut fillets treated in 2% sodium chloride brine with and without 0.05% of sodium nitrite.

Percentage of sodium nitrite	Sodium nitrite in p.p.m. of wet halibut muscle		Bacterial counts
	Immediately after treatment	After 9 days' storage at 1.5°	
None	Not appreciable	Not appreciable	166×10^6
0.05%	211	216	0.4×10^6

leptic tests were also made (table VIII). In this experiment a very marked improvement in keeping quality was obtained by the sodium nitrite treatment, as the viable bacterial counts show. Organoleptic tests by two independent judges showed that the treated fillets were much superior to the untreated ones. In this experiment the viable bacterial content of the nitrite-treated fish was apparently not high enough to cause a reduction of the nitrite.

EXPERIMENT 9

A white spring salmon (*Oncorhynchus tschawytscha*) about 1 day old in ice was filleted, and 16 pieces of muscle 5×5×2 cm. were cut from the fillets. One-

TABLE IX. Experiment 9. "Spoilage" (as judged by viable bacterial counts and organoleptic tests) and sodium nitrite content of white spring salmon fillets treated with various concentrations of sodium nitrite in 2% sodium chloride brine.

Percentage of sodium nitrite	Sodium nitrite in p.p.m. of wet muscle after 9 days' storage at 1.5°C.	Bacterial counts	Order of preference of treatment according to organoleptic tests (2 judges)	
			Fish uncooked	Fish cooked
1. None	Not appreciable	2560×10^6	4, 4	4, 4
2. 0.05%	153	125×10^6	3, 3	3, 3
3. 0.10%	400	2.2×10^6	1, 1	2, 2
4. 0.20%	780	0.66×10^6	1, 1	1, 1

litre portions of 4 different brines (table IX) were prepared, and 4 of the experimental fillets were immersed for 1 hour at 20°C. in each of them. After 9 days' storage at 1.5° the fillets were examined for numbers of viable bacteria and the amount of sodium nitrite, organoleptic tests also being made (table IX). The results show that sodium nitrite treatment strongly delayed bacterial "spoilage" in the treated fillets, especially in the higher concentrations.

EXPERIMENT 10

Twenty pieces of halibut muscle 5×5×2 cm. were cut from a fish 4 days old in ice. Four pieces were immersed for 1 hour at approximately 20°C. in 1-litre portions of 5 solutions, the composition of which is given in table X.

TABLE X. Experiment 10. "Spoilage" (as judged by viable bacterial counts and organcleptic tests) and sodium nitrite content of halibut fillets after treating in 2% sodium chloride brine with the preservatives listed.

Preservative	Sodium nitrite in p.p.m. of wet halibut muscle		Bacterial counts	Order of preference of treatment according to organoleptic tests (4 judges)	
	Immediately after treatment	After 11 days at 1.5°C.		Fish uncooked	Fish cooked
1. None	Not appreciable	Not appreciable	854×10^6	3, 3	5, 5, 5, 5
2. 0.05% sodium nitrite	163	176	66.8×10^6	2, 2	3, 3, 3, 3
3. 0.10% sodium nitrite	364	372	0.07×10^6	1, 1	1, 1, 2, 2
4. 0.20% sodium nitrite	600	710	0.019×10^6	1, 1	1, 1, 2, 2
5. 0.20% sodium benzoate	...	...	2760×10^6	3, 3	4, 4, 4, 4

One fillet from each brine was analysed for sodium nitrite content immediately after treatment. After 11 days' storage at 1.5°C. one of the remaining fillets was used in each case to determine the numbers of viable bacteria present, organoleptic tests being made on the remainder (table X). In this experiment sodium nitrite exerted a decidedly favourable effect on the keeping quality of the fish, while sodium benzoate was quite inactive in this respect, the bacterial count in this case being actually slightly higher than that obtained in the control.

B. Fillets Treated in Strong (15 or 20%) Cold Sodium Chloride Brines

In view of the facts that a brief immersion of fillets in fairly strong (15 to 20%) cold (0 to 5°C.) sodium chloride brines has the advantage of imparting a "sheen" or "gloss" to their surfaces which distinctly improves their external appearance, and that it also causes the fish to absorb sufficient salt to make it more palatable on cooking, the influence of such treatment on keeping quality, with and without added preservatives, was investigated. Experiments by one of us (P. A. S.), in connection with the mild curing of salmon, have verified the findings of Scofield (1925) that, when the "sliming" of the sides is carried

out in brine held at 2 to 5°C. the surface of the fish becomes "case hardened" resulting in an enhancement in the appearance of the cured fish. There is some indication that both sodium chloride and sodium nitrite penetrate more slowly in fish treated in cold brines, but so far the experimental evidence accumulated has been far too meagre to warrant drawing definite conclusions regarding the mechanism of this so-called "case hardening" effect and its influence on salt penetration. Experiments by Cooper and Linton (1936) have shown that treatment of fillets intended for smoking in cold brines results in a better surface sheen than when warmer brines are used.

EXPERIMENT 11

Ling cod (*Ophiodon elongatus*) about 3 days old in ice were obtained, and a number of pieces of muscle 5×5×2 cm. cut from them. Five pieces were immersed in 3-litre lots of each of two brines (table XI) for 5 minutes at 2°C., the

TABLE XI. Experiment 11. "Spoilage" (as judged by viable bacterial counts and organoleptic tests) and sodium nitrite content of ling cod fillets after different treatments.

Treatment	Sodium nitrite in p.p.m. of wet muscle immediately after treatment	Bacterial counts	Order of preference of treatment according to organoleptic tests (2 judges)	
			Fish uncooked	Fish cooked
Unbrined fillets.....	...	170 $\times 10^6$	3, 3, 3	3, 3, 3
20% sodium chloride brine	...	33.8 $\times 10^6$	2, 2, 2	2, 2, 2
20% sodium chloride brine + 0.25% sodium nitrite	269	1.18$\times 10^6$	1, 1, 1	1, 1, 1

remaining 4 being retained untreated as controls. After treatment the brined fillets were permitted to drain for 15 minutes in a 1.5°C. room, nitrite was determined in 1 of the treated fillets, and then all 3 lots were wrapped separately in moisture-proof cellophane and stored at 10°C. for 3 days. Bacterial counts and organoleptic tests (table XI) were then made, using 2 fillets for each of these determinations. The results show that a brief immersion of the fillets in cold 20% sodium chloride brine materially improved their keeping quality, and that the addition of sodium nitrite to the brine even further reduced the rate of bacterial "spoilage".

EXPERIMENT 12

Pieces of muscle 5×5×2 cm. were cut from red spring salmon (*Oncorhynchus tschawytscha*) 3 days old in ice, and from halibut 6 to 7 days old in ice. In the case of each fish 4 fillets were retained as untreated controls, and 5 were brined for 5 minutes at 5 to 6°C. in each of 2-litre portions of the solutions, the composition of which is given in table XII. After treatment the fillets were drained for 30 minutes at a temperature of about 1.5°C., one fillet from each lot being examined for sodium nitrite content (table XII) and they were then wrapped

in lots of 4 in moisture-proof cellophane and stored at 1.5°. They were examined regularly, and when they exhibited a definite "stale" odour bacterial counts were made, with the results given in table XII. It is important to note that this method of attempting to indicate a given degree of "spoilage" by the simple organoleptic test is necessarily very empirical, but it is rather doubtful if any other method would have yielded more accurate results. The criticism might be offered that it would have been more accurate to store the fillets until they had a closely similar bacterial count rather than to use this merely as a supporting test. For obvious reasons such a method presents great experimental difficulties, and it is extremely doubtful if the results obtained would have been much more valuable. Experience has shown that bacterial counts usually must vary at least ten times before very significant differences in "spoilage" are observed, as

TABLE XII. Experiment 12. Composition of brines, length of time fillets were stored at 1.5°C. before they became "stale," and nitrite and bacterial contents of fillets.

Treatment no.	Composition of the brine used and length of time the fillets were immersed	No. of days fillets stored at 1.5° before they became "stale"		Sodium nitrite in p.p.m. of wet muscle immediately after the fillets were brined		Bacterial count at the end of holding period indicated	
		Halibut	Salmon	Halibut	Salmon	Halibut	Salmon
1	Controls (unbrined).....	10	14	Not appreciable	Not appreciable	1100×10^6	506×10^6
	15% sodium chloride—						
2	5 min.....	15	16	"	"	136×10^6	228×10^6
3	10 min.....	19	21	"	"	122×10^6	236×10^6
4	+0.1% sodium nitrite, 5 min.	24	29	85	49	1522×10^6	178×10^6
5	+0.1% " " 10 min.	29	32	110	50	294×10^6	282×10^6
6	+0.2% " " 5 min.	30	33	160	61	342×10^6	624×10^6
7	+0.2% " " 10 min.	32	34	169	67	62.8×10^6	126×10^6

the experimental results in this case indicate. The combination of organoleptic test and viable bacterial count was therefore arbitrarily selected as indicating approximately the same degree of "spoilage." It will be seen from the figures given that in the case of both salmon and halibut fillets the brining treatment alone greatly increased the length of time at which they could be stored at 1.5°C. before they became "stale" and had a high bacterial content, and that the addition of sodium nitrite to the brines used caused an even more marked increase in keeping quality. It is important to note that, for a given concentration of $NaNO_2$ in the brine, very little more was apparently absorbed in 10 minutes than in 5 minutes. The salmon fillets, as might be expected from the fact that the flesh is very oily, took up much less sodium nitrite than did the halibut fillets.

DISCUSSION

The purpose of the experiments described has been to discover some method of treating fresh fillets which would improve their keeping quality, not occasion an unpleasant appearance nor in any way damage their flavour, and at the same time comply with the pure food regulations (Food and Drugs Act, Ottawa, 1938). Preliminary work was concerned with studying the effect of a number of preservatives, most of which had been previously used in fish or fish products. Many of these substances are not permitted by law in meat or fish products in this country, but they were studied partly because certain countries do permit their use, and partly because some standard of comparison was required.

It will be noticed that there was little uniformity in the bactericidal action of a given preservative in different experiments: in certain cases the effect was very pronounced, in others only moderate, while in some cases there was no observable activity. The reason for this is not clear, but it may be due to the fact that in some cases of fish spoilage the predominating organisms are more sensitive to a given germicide than in others. Thus in experiment 10 sodium benzoate was without noticeable preservative action, while in experiment 2 it was extremely effective.

That saltpetre (potassium nitrate) has been used in the curing of meats for many centuries is certain, but the exact time and circumstances surrounding its introduction are obscure. Kerr, Marsh, Schroeder and Boyer 1926; Horovitz-Vlasova 1931; Reiss, Meyer and Müller 1928; Jones 1933 and Khristodulo 1938 have all summarized the literature regarding the use of this compound in meats. From their discussions it appears that early in the present century it was made known that certain of the bacteria present in the pickles used for meat curing reduced nitrates (saltpetre) to nitrites, and that this compound in turn decomposed, yielding traces of nitric oxide which reacted with the haemoglobin of the meat to form nitrosohaemoglobin. It is this last named compound which is apparently responsible for the bright red colour characteristic of many types of cured meats (Brooks 1937). As far as can be ascertained from the literature, little or nothing is known regarding the possible bacteriostatic (or bactericidal) action of nitrites in meat pickles or in cured meats themselves. It has, however, long been known that nitrites inhibit certain of the dehydrogenase enzymes of bacteria and in concentrations of the order of 0.4 per cent entirely prevent growth of certain organisms (Stephenson 1939). One of us (H.T.) has found that sodium nitrite has a marked bacteriostatic, and, in certain instances, a bactericidal action on certain of the organisms involved in fish spoilage. This action is being studied in detail.

As far as can be ascertained from a survey of the available literature, nitrites have not been employed previously in the preservation of fresh or smoked fish, or in the treatment of fish products. In 1899, Tower (cited by Griffiths 1937) found that washing fish in 10 per cent solutions of potassium nitrate did not improve their keeping quality. Taylor (1923) stated that potassium nitrate was little used in fish curing because of the red colour it caused, and because hydrogen sulphide formation was not troublesome. He apparently believed that the

nitrates in cured meats oxidized hydrogen sulphide to water and sulphur dioxide which acted as a sterilizing and bleaching agent.

It is not yet clear why organisms associated with "spoilage" of fish muscle are so frequently sensitive to relatively small concentrations of nitrite. Tanner and Evans (1934) have pointed out that the ordinary bacterial flora of meat is inhibited by sodium nitrite in concentrations as low as 300 parts per million in pickles, while certain pure cultures of putrefactive anaerobes required very much higher concentrations to cause significant inhibition. Probably such factors as type of organism, environment (especially as regards aerobic or anaerobic conditions), ability to develop nitrite-tolerance, etc., all play some part in determining the sensitivity of bacteria to nitrites.

That nitrites are extremely effective in inhibiting bacterial multiplication in and on the surfaces of recently caught dressed fish has been shown by the writers in their experiments with ices containing sodium nitrite (Tarr and Sunderland 1939c). Nitrite ice has been found to be a very much more effective preservative than benzoic acid ice (Tarr and Bailey 1939) for dressed fish.

It has also been found that nitrites have, in many instances, a noticeable effect on the colour of treated fish muscle. Thus in certain species of salmon a more or less marked intensification of the normal red or pink colour results from nitrite treatment, the effect being apparently more noticeable in some species than in others, and seeming to vary somewhat in individual fish. In salmon this intensification of colour, though sometimes noticeable in raw fish, is more marked subsequent to heating the muscle, as in cooking, canning or smoking. Hayasi (1933) showed that haemoglobin (myoglobin) is present in the muscle of many of the species of fish which he examined spectroscopically, and it would seem highly probable that the intensification of colour effected by nitrite treatment is due to the formation of nitrosohaemoglobin, as is the case with meats. This point requires investigation. In most "white" fish nitrite treatment causes no apparent change in colour or only a faint pinkness in the muscle. Whether this incidental intensification of colour is desirable from a commercial standpoint is an open question.

Preliminary experiments made in attempts to ascertain what effect changes in pH and salt concentration of the brines may have on the efficiency of a given germicide have so far yielded inconclusive results. In the first place, although the pH of a brine may readily be made acid or alkaline, the pH of fish treated with such brines tends to remain from about 6.4 to 7.0 unless a great excess of acid or alkali is added. Undoubtedly changes in pH can be effected by adding very large amounts of acids or alkalies to a brine, but such treatment has a rather detrimental effect on the appearance of treated fillets. The study of the effect of sodium chloride concentration upon the germicidal efficiency of a given preservative is likewise complicated. The problem in this case then resolves itself into a study of the effect of the preservative in question on organisms which are and are not inhibited by sodium chloride treatment.

In closing it is necessary to call attention to the regulations governing the use of preservatives in fish and fish products. In Canada benzoates are not allowed, but sodium nitrite in concentrations not exceeding 200 parts per million

(0.02%) is permitted by law in cured meats (including fish) (Food and Drugs Act, Ottawa, 1938). Certain other countries have less stringent regulations; thus in Germany (Metzner 1933a) benzoates and a large number of preservatives not permitted in Canada can be used. Though nitrites in large quantities are undoubtedly toxic to man (Reiss et al. 1928; Behre 1939), it would appear that adults can ingest at least 200 mg. of sodium nitrite daily without ill effects: this amount would be present in 1 kg. of fish containing 200 parts per million of sodium nitrite. Also nitrites are consumed regularly in various types of cured meats without apparent injury to health.

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Occurrence and Retention of Plankton Within the Estuary

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ABSTRACT

An estuary is a region of continually changing currents and tides. Certain estuaries maintain a plankton in spite of the outflowing current of river water, either by the plankton remaining in the salt water below or owing to a mechanism which consists of an inflowing bottom current of salt water combined with a diurnal vertical migration of the plankton. Other estuaries contain only a temporary plankton.

Estuaries are bodies of water at river mouths and subject to tidal influence. There the river water mixes with sea water, particularly through tidal action and, as a consequence, salter water enters below from the sea, and less salt water passes seaward near the surface. Much of the animal and plant life in estuaries is sedentary, the association with the substratum preventing dispersal by water movements. But some forms are planktonic, even many of the sedentary forms having a free-swimming larval stage, and are more or less at the mercy of currents. How is it that estuarine plankton is not carried out to sea in outflowing river water?

Varied investigations of the estuary have been carried out in the past, but no paper dealing with the present problem has been found in the literature.

The data and conclusions in this paper are based on a study of three estuaries in eastern Canada: personal observations made on the Saint John river in New Brunswick in 1935, and on the Margaree river in Nova Scotia in 1936, 1937, and 1938; and an examination of plankton tows collected by the expedition of the Biological Board of Canada to the Miramichi river in New Brunswick in 1918. The mouths of all three estuaries are narrow and shallow.

PLANKTON TYPES

Estuarine plankton may be divided into two general types, "permanent", and "temporary". The permanent plankton is that which remains within the estuary for an appreciable length of time, irrespective of whether it is indigenous to the estuary, or introduced from outside sources. The temporary plankton consists entirely of transitory forms, (*a*) fresh water plankton carried down into the estuary in river water, and (*b*) marine plankton brought in from the sea. Its stay in the estuary is brief, and may be limited to the duration of a single tide.

THE "PERMANENT" PLANKTON

The estuary of the 400-mile-long Saint John river comprises the lower 90 miles of the river (figure 1, top). Salt water penetrates from the sea for a distance of some 25 miles (1 mile = 1.6 kilometres). An examination of the estuarine

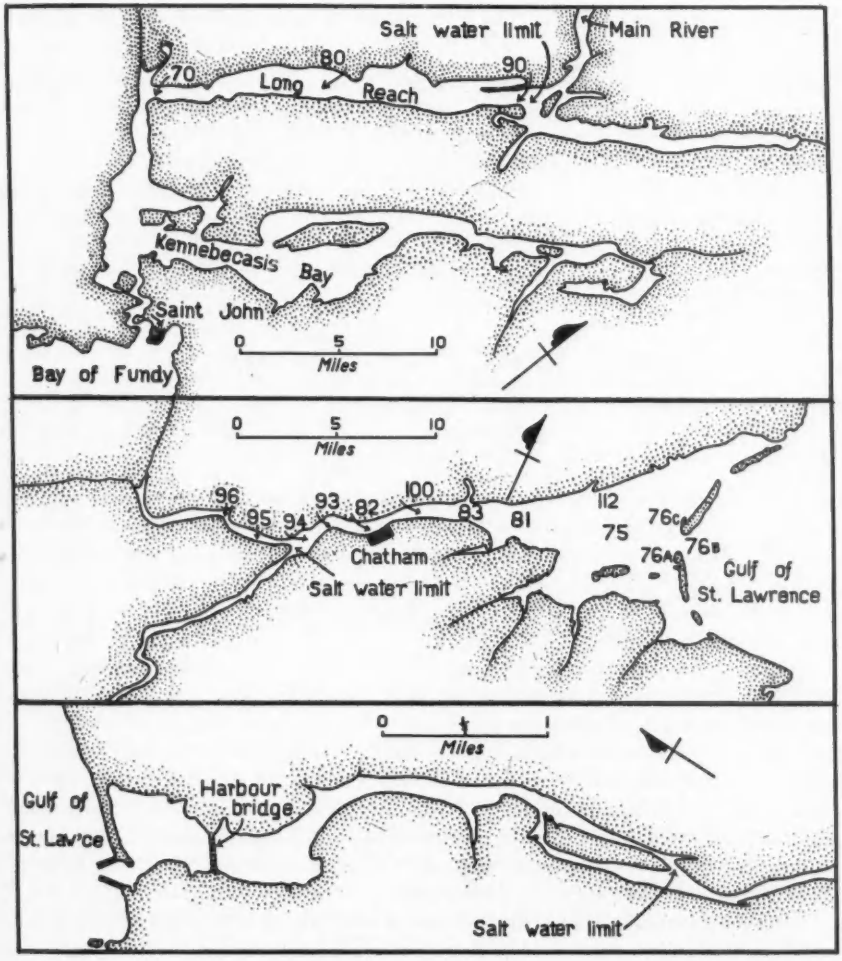

FIGURE 1. Top,—the estuary of the Saint John river; middle,—the estuary of the Miramichi river; bottom,—the estuary of the Margaree river. (1 mile equals 1.6 kilometres).

plankton on August 31, 1935, revealed the presence of numbers of barnacle nauplii and of *Sagitta elegans* near the upper end of the tongue of salt water. At station 80 (figure 1), there was a greater concentration of barnacle nauplii than

at station 70, six miles farther down stream (table I). Between stations 80 and 90, intensive mixing of salt and fresh water takes place, and at the latter station, where the water is almost fresh, very few were found. Near surface tows at stations 70 and 80 revealed a similar number of barnacle nauplii, but at greater depths they were found to be less numerous at station 70 than they were at the other station farther upstream. *Sagitta* exhibited a like distribution, though present in smaller numbers. These two forms do not breed so far up the estuary,

TABLE I. Numbers of barnacle nauplii (nau.) and *Sagitta* (Sag.) in plankton tows taken in the upper part of the estuary of the Saint John river

Type of plankton tow	Depth (m.)	Abundance					
		Station 70		Station 80		Station 90	
		Nau.	Sag.	Nau.	Sag.	Nau.	Sag.
10 minutes horizontal.....	0-1	17	3	29	2	0	0
10 minutes horizontal.....	10	..	..	125	15	4	0
10 minutes horizontal.....	20-25 (bottom)	50	8	160	22	9	1

and hence must have reached these points from outside sources. Adult barnacles occur in the lower salt reaches of the estuary as well as in the sea outside the river mouth. *Sagitta* is very common in the bay of Fundy into which the Saint John empties, and though many occur in the Kennebecasis, a branch of the lower Saint John, the absence of ripe adults in the samples taken there would suggest that they are not indigenous to the estuary. Those found in the upper parts of the estuary were all immature forms, and their presence there, as well as that of the barnacle nauplii, may be attributed to their being carried upstream by the inflowing bottom current of salt water.

Another planktont which can be readily studied is the larval form of the smelt, *Osmerus mordax*. Smelts are found in very large numbers in the 40-mile estuary of the Miramichi, a river of a total length of some 150 miles. The young are carried down into the estuary, subsequent to hatching in streams just above estuarine influence. They remain in the estuary for some time, and it appears to be the proper place for their development.

Figure 1 (middle) shows the location of stations on the Miramichi at which plankton collections were made in 1918. Table II summarizes the plankton data, comparing in chronological order catches for the upper, middle and lower parts of the estuary.

The first captures of smelt larvae were on June 4 with a 30-inch (76 cm.), no. 0 (bolting cloth standard mesh) plankton net. At this date, the larvae were from 7 to 10 mm. in length. By July 1, they had reached a length of 20 to 30 mm., and a month later measured up to 50 mm. The table suggests a gradual

decrease in the efficiency of the plankton net with seasonal progress. During the latter part of the summer, a young fish trawl was employed in taking tows, with better success.

Tows with the plankton net were of one-half hour's duration as were also those with the young fish trawl during the period of August 8 to 28, with the exception of that at station 76C, which was from 9.00 p.m. to 7.00 a.m. Young fish trawl tows during the period July 2 to 12 were of an hour's duration except for one of 2½ hours at station 94.

The majority of the tows were taken during daylight. Night tows are underlined in the table.

TABLE II. Distribution of smelt larvae in the estuary of the Miramichi river in 1918.

Type of net	Date	Depth (m.)	Stations in upper estuary		Stations in middle estuary						Stations in lower estuary				
			96	95	94	93	82	100	83	81	112	75	76C	76A	76B
30-inch No. 0 plankton net	June 4-7	0-2					20,000			112		0			
		6-10					40,000			2500		18			
	24-26	0-2	0		42	5				96		40			
		4-11	0		very many	many				many		63			
	July 1-5	0-2		– 8	850	75	0	0		9					
		6-11		4 –	200	–	–	several hundred		–					
	22-26	0-2	0	–	0		0	0		1				4	
		4-10	0	0	8		30	80		0				2	
	Aug. 9-17	0-2	0		–		0	0	0						
		8-10	–		0		–	48	0						
Young fish trawl	July 2-12	0-2		–	–	–	–	–							
		8-12		0	650	1500	100	90							
	Aug. 8-28	0-2	57		·		350		150		192	0	0		10
			–				–		–		–		–		–

Tows taken at night are underlined. All others were taken in daylight.

The "upper estuary" is that part of the river just above the region of greatest mixing of salt and fresh water, which occurs at the upper end of the tongue of salt water penetrating in from the sea. As was found for a comparable region in the Saint John (station 90), plankton was scarce in this part, as it was also in the "lower estuary" which broadens toward the coastal islands.

The greatest concentration of smelt larvae, as indicated in the table, was found in the middle estuary, and especially toward its upper end, near the limit of the tongue of salt water entering from outside. As in the Saint John, this inflowing current of salt water carries plankton with it in its flow up the estuary.

DIURNAL VERTICAL MIGRATION

A factor which appears to be of importance in the retention of certain plankton forms within the estuary is diurnal vertical migration. Smelt larvae, as do many other animals (Johnson 1938), tend to descend with an increase in light intensity, and to ascend with a decrease in light intensity. Table III sum-

marizes the data on the vertical distribution of the smelt larvae, and clearly shows the effect of light and darkness on their distribution vertically. As is read-

TABLE III. Vertical distribution of smelt larvae.

Date	Time	Station	Numbers at		Remarks
			Surface (0-2 m.)	Depth (5-11 m.)	
June 7	10.25 a.m.	82	20,000	40,000	In LIGHT more larvae in deep water
July 1	3.05 p.m.	100	none	several hundred	
June 4	3.45 p.m.	81	112	2,500	
June 4	5.30 p.m.	75	none	18	
July 26	10.10 p.m.	76A	4	2	In DARKNESS more near the surface
July 2	11.55 p.m.	94	850	200	

ily apparent, during the hours of light from 10.25 a.m. to 5.30 p.m., there were more larvae in the deep water, but in darkness (tows being taken at 10.10 p.m. and 11.55 p.m.), there were greater numbers near the surface.

In the daylight tow taken on June 7 at station 82, there were only twice as many larvae in the deep water as there were near the surface (40,000 compared with 20,000). The weather at this time was dull and rainy, and the distribution was not as distinct as might have been expected on a clearer day. The daylight tow at station 81, when 112 individuals were taken in surface water, was also taken during cloudy weather.

THE "TEMPORARY" PLANKTON

The Margaree (figure 1, bottom) is a smaller river than either the Saint John or Miramichi, brackish water being perceptible for a mere 3.8 miles above the mouth. Tows taken in the estuary of the Margaree during the summer months contained plankton forms derived from marine or fresh water habitats, depending upon the state of the tide.

At low-water, the plankton was very meagre. On June 12, 1936, a ten-minute surface tow with a 10-inch, no. 5 net, in the channel at the harbour bridge, took a very few *Daphnia*, *Cyclops*, *Diaptomus*, *Holopedium* and *Bosmina*. These were fresh water forms coming possibly from lake Ainslie, some 25 miles upstream. A rather intensive mixing of surface and bottom water occurs at this point so that little difference would be expected in the nature of the fauna at various depths. Other tows at low water were of a like nature.

At high tide, the plankton was of a marine character. Surface tows taken in June, 1937, at the bridge captured the cladocerans *Evadne* and *Podon*, the copepod *Calanus finmarchicus*, polychaete larvae, ctenophores and medusae.

This examination of Margaree plankton shows the difference in character of plankton forms taken at opposite points in the tidal cycle, and suggests that a permanent plankton comparable to that found in the other two rivers does not exist in this estuary.

ESTUARINE CURRENTS

As already indicated in the definition of the estuary, a tongue of salt water extends up river under the upper layer of lighter fresh water flowing out on the surface. Were there no mixing between the salt bottom layer and the lighter body of fresh water flowing out over it, the salt layer would not circulate but merely move to and fro with the tide. Owing to the fact that some of the salt water mixes with the fresh upper layers, it leaves the estuary as brackish water. This results in a continual depletion of the salt water, and hence there is an inflow

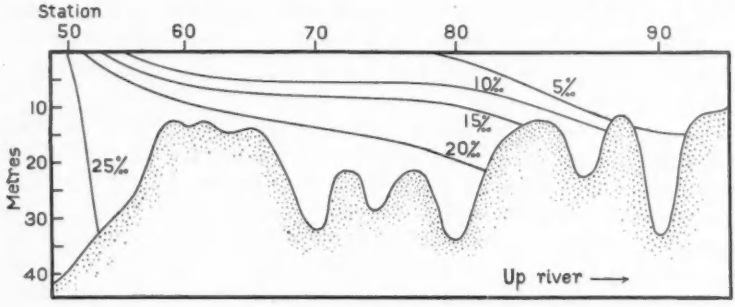

FIGURE 2. Salinity of the Saint John river, August, 1935.

of salt water under the outflowing upper fresh water. This inward current may be illustrated by the salinity chart for the Saint John (figure 2). A similar state occurs in the Miramichi.

Owing to the limited length and shallow nature of the Margaree, tidal effects in this estuary are more pronounced than in the Saint John or Miramichi. Unlike the latter estuaries, no particular body of water in the estuary of the Margaree remains there longer than the interval that elapses between two tides, that is, water that enters the estuary from the sea on a flood tide leaves again on the following ebb. The estuary is so short and so shallow that ample opportunity is given for this to occur. The average velocity of the outflowing water is approximately one mile (1.6 km.) per hour. But salt water penetrates at the most only four miles, so that the velocity of the outflowing water during the six-hour period of the ebb tide is sufficient to carry all of the water in the estuary out to sea. At the end of the ebb, the water leaving the mouth of the river consists almost entirely of fresh river water with little salt water remaining in the bottom layers.

In a longer estuary such as the Saint John or Miramichi, water at the upper end of the estuary carried down stream on a falling tide does not reach the river mouth before the turn of the tide; hence it flows back upstream on the flood. The result is that the tide in long estuaries does not interfere with the net inflow of salt water occasioned by estuarine mixing.

DISCUSSION

Since plankton is at the mercy of water movements (Welch 1935, p. 204), it is self-evident that the distribution of plankton within the estuary may be accounted for to some extent by currents. In the Saint John, the inflowing bottom current carries the *Sagitta* and barnacle nauplii toward the upper end of the estuary. A piece of inanimate matter after reaching the region of mixing toward the upper end of the estuary would be expected to mix finally with fresh river water and leave the estuary in the upper outflowing current. Some members of the incoming plankton follow such a course. *Sagitta* and barnacle nauplii occur in the upper outflowing water of the Saint John river. But there is apparently a tendency for these forms to avoid the fresh water layers, for the concentration of these forms increases in the bottom layers as one proceeds up the estuary, the numbers in the surface waters being comparatively few. Additional data are required to determine the factors involved in this apparent negative reaction to the fresh water layers.

In the case of certain marine or brackish water forms staying mainly in the bottom salt water, a simple inflowing bottom current may be quite sufficient to ensure maintenance within the estuary. But where smelt larvae enter the estuary in the fresh water flowing into it from the river, some additional factor must be involved whereby the larvae descend into the inflowing layer of salt water, for if they were to remain in the upper layer, they would be carried from the estuary. This factor appears to be diurnal vertical migration, the larvae being negatively phototropic. Since daylight is of a longer duration than darkness at this time of year, there being an average of 15 hours between sunrise and sunset during June and July, the larvae spend more time in deep water. Their presence there in the inflowing current assists in maintaining the concentration well within the estuary.

In a river which is without a net inflowing bottom current, the benefit of the sojourn in deep water is lost. The Margaree estuary is without such a current owing to its shallowness and limited length. The lower tongue of salt water is carried out of the estuary almost completely with each ebbing tide before the commencement of the flood, carrying the contained plankton with it. Smelts spawn in the Margaree in appreciable numbers, but apparently the young are carried out of the estuary on the ebbing tides subsequent to hatching, since none have been found there. There is no retention of plankton as described for the other two estuaries.

CONCLUSIONS

A characteristic feature of estuaries is an inflowing bottom current of salt water. Plankton is carried by this current up the estuary. The bottom current

may be interrupted by tides, so that on the ebb its direction of flow is reversed. The effect of this backward flow in the ultimate retention of plankton within the estuary depends upon the velocity of its flow downstream in relation to the length of the estuary.

In short estuaries, the configuration of which is such that the bottom salt water leaves the estuary on each ebbing tide, there can be no permanent plankton. Plankton forms present come from outside sources.

In longer estuaries where the bottom water during an ebb tide does not flow back sufficiently far to leave the river, this water returns on the flood so that the two tides balance one another. Hence the net inflow of salt water resulting from the mixing of salt and fresh water within this type of estuary is not affected by the tidal cycle.

The net inflowing bottom current alone is sufficient to retain within the estuary plankton which stays mainly in the salt bottom water. Plankton in the upper outflowing layers will leave the estuary unless its stay in this outflowing current is interrupted by a longer sojourn in the lower inflowing water. A diurnal vertical migration of the plankton brings this about, and, along with the inflowing bottom current, results in the retention within the estuary of plankton with such migration.

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"Sea Lice" (*Lepeophtheirus*) and Death of Salmon

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ABSTRACT

In 1939 *Lepeophtheirus salmonis* infected salmon entering Moser river, Nova Scotia. Some fish had much of skin in occipital region removed and died some time after entering fresh water. It is suggested that these copepods cause the condition on the top of the salmon's head known as "white spot."

Lepeophtheirus salmonis is reported (Scott and Scott 1913; Wilson 1905) as occurring on Atlantic salmon throughout its range from Maine to Labrador on

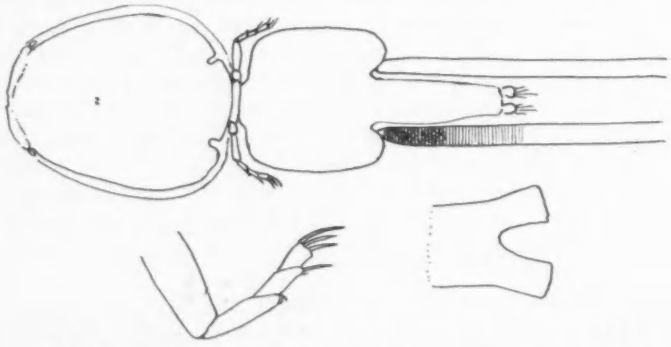

FIGURE 1. *Lepeophtheirus salmonis* from Moser river, N.S. Above, female, body length 11 mm., and eggs sacs (only partly shown) 17 mm. long; below, fourth leg at left and sternal fork at right.

the east coast of North America. We have observed what seems to be this species on salmon in Apple river at the head of the bay of Fundy, in the Margaree river of the western coast of Cape Breton island, and in the Moser river of the outer coast of Nova Scotia. Specimens from the latter locality are found to agree with Wilson's (1905) figures, except in the shape of the sternal fork (abruptly truncated rather than oval and pointed), and in the presence of a small curved seta on the first joint of the exopod of the fourth leg (figure 1). In the

same respects as well as in some others, they differ from the figures of Scott and Scott (1913).

Salmon were trapped when ascending the Moser river during the summer of 1939, and all either carried "lice" or had scars caused by "lice". The earliest running fish were but slightly infested and showed no noticeable abrasions, but as the season progressed the "lice" became more numerous and the abrasions more severe. The larger salmon were not as heavily infested as the grilse. The infestation apparently reached its peak about the middle of August, both in numbers of lice carried and in the severity of the abrasions. At this time fish, which were apparently freshly ascended from the estuary, carried hundreds of lice ranging in body length from 3 to 12 mm. Some of the grilse had an almost complete layer of lice extending from the posterior edge of the eyes to the caudal peduncle on the dorsal part of the body with also a few lice around the anal and pelvic fins. As *Lepeophtheirus* drops off the salmon a short time after salmon enter fresh water, the numbers of lice which had infested some of the salmon which had lingered in pools below the trap could be judged only by the extent of the abraded areas. The last fish bearing lice were taken in the trap on September

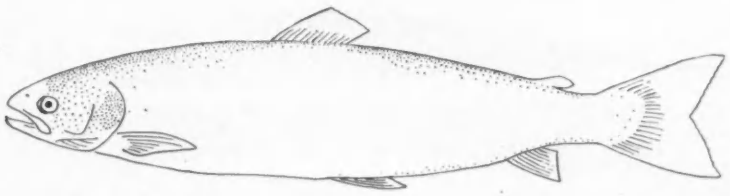

FIGURE 2. Salmon with distribution of "sea lice" shown by dots.

29 but these had evidently not been as heavily infested as those taken early in August.

DAMAGE BY THE "LICE"

No appreciable damage to the salmon was noticed at Apple river, and the amount in the Margaree district was negligible as observed during the salmon investigations there.

A grilse hooked at the upper end of the Moser estuary on August 5, 1939, was found to be so heavily infested with *Lepeophtheirus* that its body colours from above were obscured by the brown parasites. The lice covered the head from the eyes backward and extended downward over the opercula, and even the posterior part of the eyes was covered. The areas commonly parasitized on the grilse are shown in figure 2.

Salmon ascended the lower part of the river on August 6 and quite a number of them died during the afternoon, evidently as a result of the exceptionally high temperature (29.2°C. at 1.30 p.m.). About a dozen of the dead salmon were examined and all showed light-coloured abraded areas caused by the "lice". Doubtless this contributed to their death.

DEATHS FROM THE "LICE"

Practically all the salmon running during July and up until August 6 were very badly infested with the parasites and the later ones showed white patches (1) over the frontal region, (2) on the opercula, (3) along the occipital region and (4) extending posteriorly along the nape. These abrasions did not appear to be severe enough of themselves to cause death.

Some days after the "run" of salmon on August 6, Mr. Carver, a resident of Moser River village, stated that he was able to see fish at Gaspereaux falls, some little distance up the river, very plainly and that some of them had "bright red heads". We were unable to account for his observations as no salmon had shown such a condition when passing through the traps. However, a few days later a dying grilse found above the racks showed the condition which had been described. The skin from the nape, the occiput and part of the frontal region had sloughed away revealing the naked reddish flesh beneath. In the water this appeared very red and could be seen for a considerable distance. Since the "lice" drop off the fish a short time after the salmon enter the river, it seemed evident that the

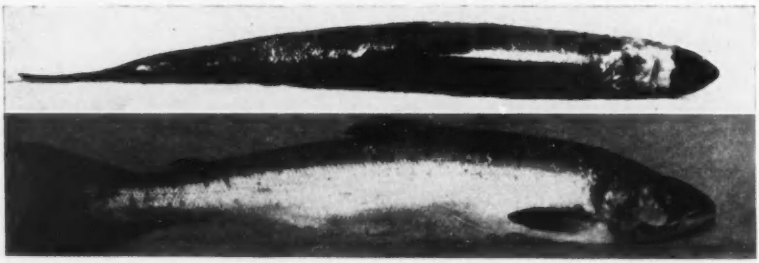

FIGURE 3. Badly infested grilse showing abraded skin along dorsal areas and on operculum, also deep lesion extending posteriorly from the medullary region.

missing skin had not been eaten away by the parasites. We found that on many of the fish which were severely infested the skin was loose over the same areas and when scraping "lice" from these fish the skin readily came away revealing the flesh beneath. For about two weeks after this condition was first noticed, ascending grilse were found in this condition (figure 3) and a few dead or dying fish lodged on the racks and others were found along the stream.

CALDERWOOD'S "WHITE-SPOT" DISEASE

Before the skin sloughs away there is a distinct white area over the regions mentioned above and Calderwood (1905) has described a condition of this kind as "white spot". His entire description leaves little doubt that this is identical with the conditions found on Moser river salmon. Since it was found by him to be associated with very low water when the salmon were detained from ascending the streams and were congregated near their mouths, he attributed it to injury from sunlight. In his article no mention is made of the parasites and since the

specimens he examined had been sent to him, he had no opportunity to associate the abraded areas and lesions with *Lepeophtheirus*. The loosening of the skin, however, might be associated with light injury, as the infested fish swam with this area at surface level, or the loosening might be associated with some osmotic action. A very noticeable condition in the affected fish was a distinct cavity in the frontal region caused by the atrophy of connective tissue normally situated between the skin and the frontal bones. Fish taken during September and October which had completely recovered from the infestation had a depression in the frontal region.

This "white spot" condition has previously been observed in Canadian waters, heads of salmon having been sent for examination to the Atlantic Biological Station in 1929 from various points on the coast from the St. Lawrence to the Saint John (Huntsman 1930), which were diagnosed by M'Gonigle (1931) as "white spot" disease.

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Life History of Sea-Running Brook Trout (*Salvelinus fontinalis*) of Moser River, N.S.

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ABSTRACT

Smolts, kelts and non-mature large fish descend in May and early June to the sea, to remain feeding in the estuary or near the shore. They began (1939) to ascend the river in late June, and 93% of the "run" was in July.

Fish marked when descending one branch of the river ascended both that branch and the river above but the proportion of marked fish was greater in the branch.

These trout agree with the local salmon in having smolts that become silvery before migration and that are two or three years old. Trout smolts and kelts remain in the sea only about two months before returning to fresh water.

In various species of salmonids which have access to the sea, some individuals descend to the sea where they spend a part of their life, whereas others complete their entire life cycle in fresh water. Whether fishes which behave so differently constitute separate populations with genetic differences, or whether the differences in behaviour are merely manifestations of a wide range of behaviour within a single species has been a subject of speculation among ichthyologists.

On our Atlantic coast some of the speckled trout (*Salvelinus fontinalis*) descend to the sea, whereas others remain in the streams or lakes. Those which descend to the sea and become the silvery "pink fleshed" fat trout before returning to the rivers are the well known "sea trout" of our Maritime provinces. There, the "sea trout" is considered a very important game fish. It is generally taken by angling with artificial flies both in the streams and in the sea.

Taken in the sea or soon after returning from the sea, the "sea trout" are very different in appearance from the fresh water trout. Although more slender than the latter, they are relatively thicker through the body. The dorsal surface is a dark greenish-blue colour, visible through a thin coat of silvery guanin crystals. The sides are very silvery or with pearl-like iridescence and sometimes with a few pink spots discernible through the guanin. The belly is pearly white.

Systematists have for obvious reasons been uncertain regarding the status of the sea-run trout. Jordan and Evermann (1902) do not mention it in their description of the brook trout but state regarding charrs in general, "sometimes descending to the sea where they lose their variegated colors and become nearly plain and silvery".

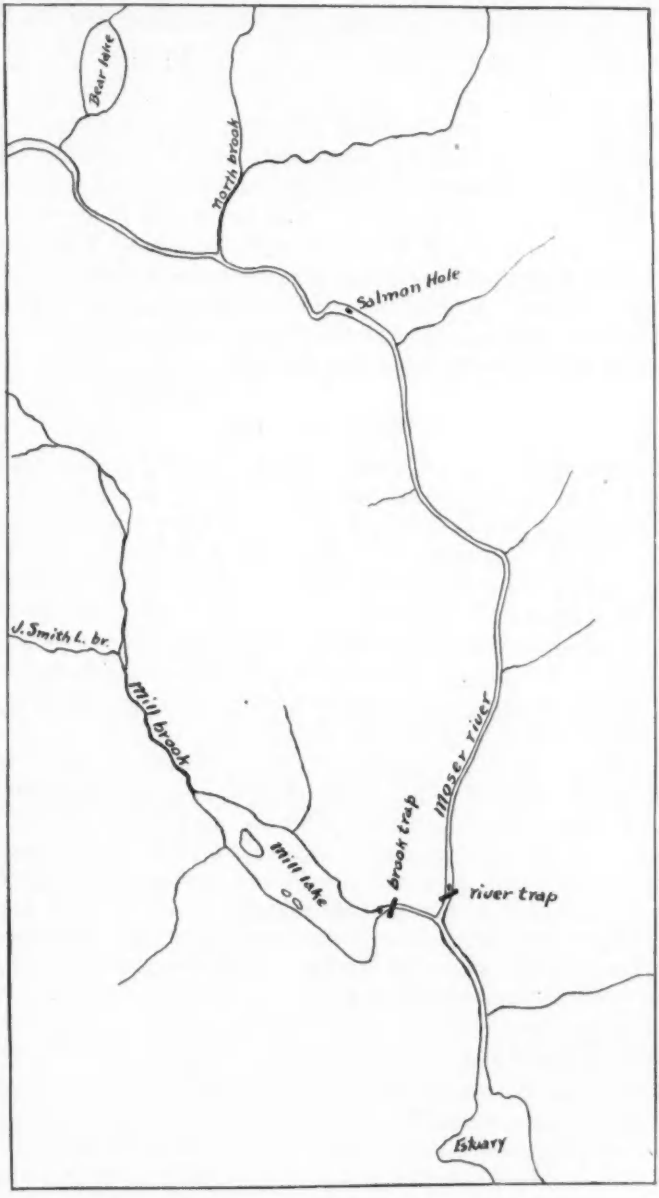

FIGURE 1. Lower part of Moser river with tributaries.

Although Hubbs (1926) included the "sea trout" as *S. fontinalis hudsonicus* (Suckley) in his check list of the fishes of the Great Lakes, in a recent key of Hubbs and Lagler (1939) it is considered a doubtful sub-species.

THE TRAPS

In 1939, fish traps were installed at Moser river (1) on Mill brook flowing from Mill lake and (2) on the river a short distance above the mouth of the brook (fig. 1). They consisted of slat barriers with the pounds arranged to capture separately the upward and the downward migrating fishes. These traps were similar to others used at the Apple and Margaree rivers (White 1939). Although primarily for the study of the salmon, they furnished material for studying the sea-running trout. That on the brook was in operation from May 2 to November 1, and that on the river from June 3 to October 24.

SEAWARD MIGRATION

On the first night, May 2-3, that the brook trap was in operation, 58 trout were taken in the pound which retained the downward migrating fishes, and on the second night 103, the maximum number taken. The downstream migration from Mill lake continued until June 12 and during this migration 1,220 trout passed through the trap. Over 50% of the fish taken were caught in the period May 10 to 22, and less than 2% in June. That a part of the migration had passed before the traps were installed is indicated by the abrupt start of the catch to be seen in figure 2. There is some indication of correlation between the daily runs and increasing temperature as represented by morning records (fig. 2), but light intensity, which was not recorded, was also an important factor.

All the trout taken on their seaward migration in the brook trap were marked by shaving off the adipose fin with a razor blade, an operation that leaves a clean scar which is probably not duplicated by natural mutilations.

Owing to the fact that the barrier and traps on the river were not placed until some time later than those on the brook, only a small percentage of the run on the river was caught, and those taken were liberated unmarked.

The behaviour described is in contrast with that given by Bigelow and Welsh (1925), who state that "on Cape Cod the sea trout go down to salt water in November immediately after spawning."

Character of Fish

Late in the fall of 1938, we fished Mill lake with set lines, even after the ice had formed in mid-November, and among the fishes taken were a considerable number of trout of various sizes. These were very dark trout with no evidence of silvery coloration but from their general form we had believed them to be sea trout. In the spring, trout of similar sizes were taken in the brook trap, but instead of being dark they were in various stages of becoming silvery and these silvery fish were found to actually consist of the three groups which follow.

SMOLTS

There has been no recognition of a smolt stage for this species. However, the smaller fish of the run resembled salmon smolts in their form and silvery colour, and also in showing in their scales none of the rapid growth that seems definitely associated with a sojourn in the sea. Dr. W. S. Hoar made a study of our collection of scale samples, made at random during the run, and found that 79.4% of these fish were migrating as 2-year and the remainder as 3-year smolts. The average lengths of the two groups were 17.0 and 19.7 cm. respectively.

KELTS

Among the larger fish some had spawned the previous fall but were already becoming silvery or had become so, thus warranting the term of mended kelts. Those which had migrated to sea as 2-year smolts, as shown by the scales, are stated by Dr. Hoar to have averaged 23.8 cm., while those that had migrated after three years averaged 28.9 cm.

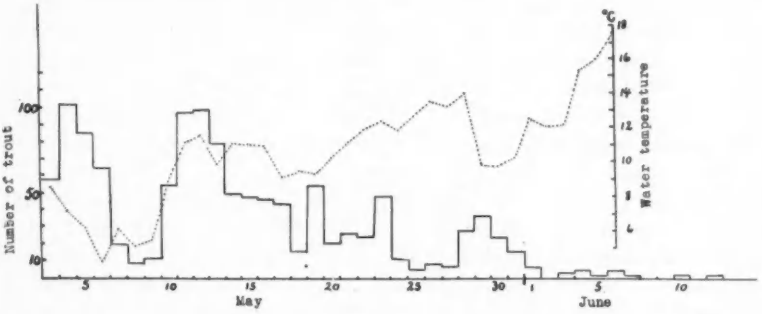

FIGURE 2. Daily numbers of trout trapped when descending Mill brook. Water temperatures as taken daily at 8.00 a.m. are shown by the dotted line.

NON-MATURE LARGE FISH

Among the larger fish were some which on examination were found to be sexually immature and not to have spawned the previous fall. Such fish are known to constitute a considerable portion of the trout ascending rivers from the sea during the summer, as mentioned by Huntsman (1938). They were not readily separated from the kelts, either in external appearance or in the condition of the scales, although Dr. Hoar stated that scales from some of the larger fish had markings which might be spawning marks.

RECAPTURES IN THE ESTUARY

Immediately after we commenced marking the trout we began getting reports of the taking of marked trout in the estuary. We examined the catches of a number of anglers fishing there and in them were a number of the marked trout. On May 15 and 16 we fished with hook and line in the salt water of the

estuary and took twenty-two trout. Nineteen of these were smolts and three were mended kelts. Eleven of these trout were fish which had been marked at the brook trap. The trout taken in the estuary at the time consisted of smolts, mended kelts and non-matures, i.e. fish which, although in the river from the previous summer, had not spawned and were on their second seaward migration.

Apparently the trout pass out of the upper estuary very soon after descending the river for at no time did there appear to be any concentration of trout in that part of the estuary, and also the catching of trout by anglers ceased abruptly after the run of seaward migrating trout had ended. We did not hear of any trout being taken in the estuary during the rest of the summer.

SEA LIFE

Very little is known concerning the life of these trout while in the sea. At some places along the coast of the Maritimes they are caught in shore fish traps and nets. In this locality they have been taken during the early summer by angling outside the estuary and have been observed in small schools in the shallow waters around the inner islands. The data we have indicate that their range in the sea is confined to the shore waters and the estuaries. This agrees with the statements of Bigelow and Welsh (1925) for the gulf of Maine. "Trout never stray far from the stream mouths in the Gulf. So close indeed do they hang that we have never heard of the capture of a single one outside the tidal creek or estuary into which its home stream empties."

In the bay of Fundy this condition seems more extreme, perhaps owing to the heavy tides quickly removing the estuarial water. Perley (1852, p. 198) reported that the species had not yet been seen in that bay, "which it is supposed not to frequent" and Huntsman (1922, p. 12) stated that he had never obtained it in the salt or brackish water around the bay. However, I have taken it in the upper parts of river estuaries at the head of the bay.

This is in striking contrast with what is found in the gulf of St. Lawrence. There the sea trout are taken on the coast in nets as we have observed on the west coast of Cape Breton island and as has been reported for Tabusintac, N.B. (Perley 1852, p. 72), Gaspé peninsula, Quebec (Bell 1859, p. 206), Magdalen islands (Perley 1859, p. 97), and west coast of Newfoundland (Reeks 1871, p. 2555).

RETURN FROM THE SEA

General

The first trout of the run from the sea was taken in the traps on June 17 (fig. 3) and four others during the remainder of the month. There was a small run between July 1 and 9 when 53 trout were taken in the traps. They returned for the most part during July when 93% of the run occurred. In the 9 days from July 14 to 23, 74% of the catch was taken in the traps. There was a total of 688 taken and of these 602 were taken in the river trap and 86 in the brook trap. It will be noticed in the figure that there is a good correlation between rises in water height and increases in the run, but it is also evident from the figure that

increases in the run are correlated with rain, and indeed it is true that the increases began even before the water had risen. It will also be noted that some of the latest running trout ascended when the stream level was at its lowest.

All the late-running trout were heavily infested with parasitic copepods which have been tentatively identified as immature *Lepeophtheirus salmonis*. No adult female parasites with the long egg strings such as were common on the salmon were observed on the trout.

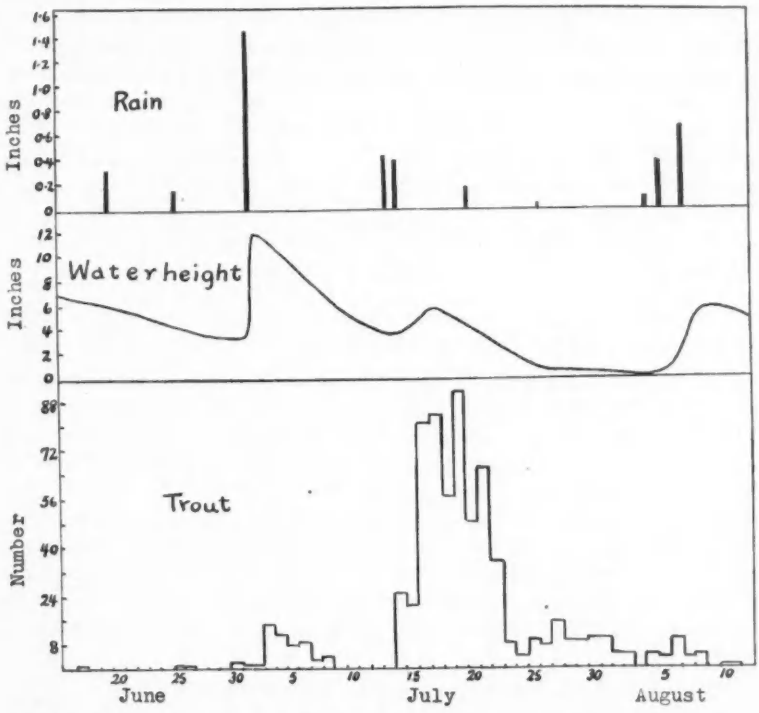

FIGURE 3. Daily numbers of trout trapped when ascending the river and Mill brook, with rainfall and water height also shown. 1 inch = 2.54 cm.

THE MARKED TROUT

The first marked trout returning from the sea was taken in the brook trap on July 2 and the second the following day in the river trap. During the run 31 of the marked fish entered the brook trap and 96 the river trap.

Although more of the marked trout ascended the river than returned to the brook, it is significant that a higher percentage of marked fish than of unmarked fish entered the brook both early and late in July (34.3% compared with 11%, and 14.3% compared with 9% respectively). This indicated a tendency of the trout to return to the branch from which they had migrated. No trout entered

the brook during August and the percentages of both marked and unmarked fish entering the brook decreased rather steadily throughout the season as if the brook were becoming comparatively less favourable for trout.

After ascending the river above the trap, none of these trout, as judged by angling, remained during the summer in that part of the river below the Salmon hole, which is some three miles (5 km.) above the trap. During September, we found there both marked and unmarked trout.

During high water in October adult trout passed down through the river trap and then up through the brook trap. Among these fish were both marked and unmarked trout. Some of this run which were tagged with nickel wire at the down trap on the river were retaken within a few hours in the brook trap. Thus the definitely known migration of the marked fish which were later also tagged was down Mill brook in May and then to the sea. During July they ascended the river and passed up through the river trap, continuing their ascent to pools above, where they remained until October. Then they descended the river and ascended the brook.

We presume from the position of their known spawning grounds that they passed through Mill lake and ascended the brook above the lake.

FATE OF MARKED FISH

Of the 1,220 trout marked on their descent from the brook only 127 or 10.4% were retaken in the traps on their return from the sea. The first decrease in their numbers of which we have knowledge was from anglers fishing in the estuary. However, it is estimated that they could not have taken more than a hundred and fifty of the marked fish.

Other decreases in the numbers of the trout may have been caused by fish-eating birds in the estuary or among the coastal islands, or by seals and fishes. Cormorants were abundant in the vicinity as there was a breeding colony on Little White island not far away. We observed them feeding in the estuary during the descent of the smolts. Small flocks of mergansers were in the estuary at the time, and analyses of the stomach contents of birds taken late in the season up the river showed that they will feed upon trout of considerable size.

The returning trout showed scars of several types, two of which were recognized as those caused by eels and by great blue herons. The latter fed regularly at low tide in the estuary. Several of the trout had scars with which we were not familiar.

Another reason for the small number returning to the Moser river may have been that they entered other streams. Since they showed only a partial discrimination between the river and the brook they may have entered other streams along the coast.

It is estimated that about three hundred of the returning sea trout were taken by anglers below the traps and as 16% of those examined were marked fish this would indicate that about fifty were among their catches.

Angling both in the spring of the year and in the summer during the return of the trout could not have accounted for more than 16% of the marked trout.

MATURITY AND SEX

We have previously in this paper made reference to non-mature fish or those that do not spawn the year they return from the sea. The stages of maturation of the gonads were noted for the 48 trout taken during September in the Salmon hole, at which time those which were maturing for spawning were readily recognized. Of the 35 females only 18 were maturing, and of the 13 males only 3 were maturing.

In the European sea trout of the genus *Salmo*, similarly some of the fish that return to fresh water are sexually immature (Menzies 1936, p. 97).

Twenty-three smolts were examined for sex determination and 18 or 78.3% were females, and of 75 trout examined on their return from the sea 59 or 78.6% were females.

FOOD

We have only a few food analyses and observations on the feeding habits of the "sea trout", but these aid in understanding their life-history.

Fingerling Parr

We have been unable to distinguish between the young of the "sea trout" and those of the purely fresh-water trout. However, by collecting the younger stages near where many "sea trout" spawn and where "river trout" are scarce, we feel reasonably certain that the young trout taken were mostly those of the "sea trout". Where the young of the two occupy the same habitat it is improbable that there is any difference in their feeding habits.

In the fall of 1938, many "sea trout" were observed spawning in Johnny Smith lake brook, which empties into Mill brook. On June 13, 1939, nine fingerlings, ranging in length from 30 to 48 mm., were collected near the spawning area. The nine stomachs contained 263 recognizable food organisms. Of these 240 were chironomids (midges) which consisted of 194 pupae, and 46 larvae. The pupae were all of very small forms. Chironomids constituted on the average 78.1% of the food. Other forms taken were Trichoptera (caddis worms), Ephemeroptera (may-flies), Simuliidae (black flies), and several terrestrial forms.

Six trout fingerlings taken July 14 from the lower part of Goldmine brook, a small tributary of Mill lake, contained 91% chironomids which also were mostly in the pupal stage.

Older Parr

On June 13 the stillwater at the mouth of Johnny Smith lake brook contained a large number of trout of the yearling and 2-year-old classes. Many of these fish were of sizes which would migrate as smolts the next year. Six of these, ranging in length from 14 to 18 cm., were taken on an artificial fly and preserved for food analysis. As the flies used were rather large, none of the smaller fish were taken.

Their stomach contents show that they were actively feeding and had taken a wide variety of insects as well as snails. Five of these fish had taken Tri-

choptera, which made 47.5% of all the food. Three fish had eaten snails, which constituted 27.5%. Chironomid pupae occurred in two trout and made 8.3% of the food. The remaining 16.7% consisted of Ephemeroptera, Plecoptera (stone flies), *Simuliidae* and a number of terrestrial forms.

Smolts

During the smolt migration, the fish were rising for food above the traps. This period of feeding was revealed also by the presence of new growth on their scales. Six smolts taken in Mill brook on May 9, ranging from 16 to 22 cm. in length, were well filled with food. Chironomid pupae, including a number of medium-sized species, constituted 79% of the food. Other forms taken by these fish were Odonata (dragon flies) 11%, Trichoptera 8% and *Simuliidae* 2%.

On May 16 to 17 smolts were taken on a baited hook a short distance below the head of tide. Fish which had been marked at the brook trap were among these. The stomach contents of seventeen that had been marked at the brook trap and that ranged in length from 17 to 23 cm. were examined. Although the point at which these fish were taken is some distance below the head of tide, there was considerable river water above the salt water and this layer undoubtedly carried some fresh water organisms. Some of the fish taken at this place may have been very recent migrants from the river, and thus with food in their stomachs not that of their immediate habitat.

The food found reflects the transition from the fresh to the salt water habitat. Nineteen young eels (elvers) fresh from the sea and still transparent were found in the stomachs of five trout and constituted 30.2% of all the food. Salt water isopods (*Idotea phosphorea*) were next in bulk, being 11.5%. Plecoptera made up 10.8%, and salt water amphipods (mostly *Gammarus locusta*), 10%. Other forms constituted percentages as follows: Trichoptera 5.4%, Ephemeroptera 5%, small salt water shrimps 1.5% and miscellaneous organisms 25.6%. The latter included *Nereis* (sand worms), *Simuliidae*, Odonata, *Sialis* (alder flies), *Chironomidae*, Coleoptera (beetles), *Formicidae* (black ants) and fish,—*Gasterosteus* (stickleback) and scales of *Pomolobus* (gaspereaux).

The finding of elvers was of particular interest, since many previous examinations of trout from fresh water habitats where small eels occur had never revealed any elvers in their stomachs. Several of the trout contained partly digested fresh water forms in the pylorus of the stomach while the cardiac part contained freshly ingested salt water forms, which probably represented their first salt-water food.

In the Sea

We have had no specimens of trout from the outer estuary or farther out in the sea in the Moser river district. However, the marine forms found in the stomachs of those taken in the upper estuary may be indicative of the types of foods taken in the sea, i.e. crustacea, fish, sandworms, etc. According to Bigelow and Welsh (1925) they "feed chiefly on shrimps, mummichogs (*Fundulus*), and other small fish."

After Return from the Sea

At Moser river the "sea trout" return mostly during the month of July, which is much later than for cape Cod (April to mid-May) as given by Bigelow and Welsh (1925). Although the ascending fish rise readily to an artificial fly, no food was found in their stomachs. After ascending the lower part of the river they congregate in the various stillwaters. Trout taken at the Salmon hole during September contained insignificant amounts of food in their stomachs and their intestines were empty. The contracted condition of the stomach and the intestines indicated that they had been fasting for some considerable time. That they do little active feeding after returning from the sea is substantiated by the fact that there are no narrow circuli on the scales, such as characterize growth in the river. Dr. Hoar in his study of trout taken during their seaward migration in May found that the scales of smolts showed both winter and spring growth for the period preceding their capture, but kelts and non-mature fish showed neither winter bands nor other growth subsequent to the formation of the broad circuli of sea-growth for the previous year. Moreover, scales which had two bands of sea growth showed nothing indicating river growth between the bands of sea growth.

Kelts

Four kelts taken at the extreme head of tide on May 6 had been actively feeding upon stream insects. These fish ranged in length from 30.5 to 43 cm. and contained 55 Trichoptera (72%), 22 Plecoptera (22%) with the remaining small percentage made up of immature Ephemeroptera, *Tipulidae* (craneflies), Odonata and *Corixidae* (water boatmen). .

A female kelt 34 cm. long, taken below the head of tide on May 16, contained 26 elvers and 6 isopods (*Idotea phosphorea*).

COMPARISON OF "SEA TROUT" WITH SALMON

Spawning

The trout spawn at Moser river during October in gravel areas which have spring seepage. The salmon spawn during late October and early November on gravel rapids without spring seepage. The "sea trout" fry emerge during April and early May, the salmon fry during June.

Growth in Stream

In Mill brook, where comparative measurements were made, the trout at the time of migration averaged 17.5 cm., and the salmon 16.1 cm. Both migrate after two or three years in the river (rarely longer), in 1939 79.4% of the trout and 81.6% of the salmon migrating after two years and both becoming silvery before migrating to the sea. Trout smolts migrated during April and May, the migration reaching its peak before the middle of May. The salmon smolts migrated during late May and early June, the migration reaching its peak the last week in May. Salmon smolts and trout smolts were taken together in the estuary.

Growth in the Sea

After their first migration, the trout return to the river as fat fish after about two months in the sea whereas the salmon spend more than a year in the sea. Both trout and salmon kelts return as fat fish after two or three months in the sea, the period for the salmon being the longer.

Behaviour after Returning

Both trout and salmon returning from the sea are largely females and are well silvered on their return. They assemble in the deep pools or stillwaters where they remain until near the spawning season. Both lose the guanin coat and become dark. They feed sparingly in the river prior to spawning and remain throughout the winter in the fresh water. Salmon and trout kelts become silvery again before returning to the sea during April and May.

From the above comparison it will be seen that there is great similarity in the behaviour of the trout and salmon in the Moser river, the greatest difference being in the shorter periods spent in the sea by the virgin trout and the smaller growth of the trout in the sea.

Some of the trout spawn the fall of the year in which they descend to the sea as smolts, while others are like the salmon grilse in spawning only the second fall after descent. However, these latter differ from salmon in spending in the river instead of the sea the time between successive seasons of growth.

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Specificity of Triamineoxidease

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ABSTRACT

The substrate-specificity of a newly described enzyme common to six bacterial species, comprising five different genera, isolated from such widely divergent sources as decomposing fish, well-water and surface taint butter, was investigated. Of the various substrates studied only trialkylamine oxides having the general structure $R_3 \equiv N = 0$ were activated with subsequent reduction, the corresponding volatile base being formed in each case. Betaine, choline, acetylcholine, ergothioneine and stachydrine containing an atomic group similar to the above were not activated. The designation "triamineoxide*ase*" is proposed for this enzyme.

In previous papers the writer showed that certain bacterial cells possess an enzyme which activates trimethylamine oxide rendering it capable of reduction by many dehydrogenase systems of lower potential level. Working independently, Watson (1939, a and b) came to a similar conclusion, and stated that, "From these results it is evident that trimethylamine oxide, like nitrate, requires a degree of activation by the bacterial cell." The writer (1939, a and b) suggested that the activating principle be named "Trimethylamineoxide*ase*". In this paper reasons are given for substituting the more general and concise designation "Triamineoxide*ase*" (Tarr 1940) for the enzyme. In previous work (Tarr 1939b) an attempt to obtain a cell-free preparation of the enzyme by an autolytic method was unsuccessful, and therefore in the experiments to be described intact bacterial cells have been employed. It is possible that a cell-free preparation of the enzyme could be obtained using a wet crushing mill of the type described by Booth and Green (1938).

EXPERIMENTAL

Cultures

The following organisms employed in previous experiments (Tarr 1939b) were used: Cultures nos. 1 (*Micrococcus*), 18 (*Micrococcus*) and 22 (*Achromobacter*). Cultures C2, C8 and 4 were obtained from Professor Eagles of the University of British Columbia. They possessed the following characteristics: Culture C2, isolated from butter with "surface taint" and capable of producing this defect experimentally; member of the genus *Escherichia*. Culture C8, isolated from a similar source and capable of producing the same defect in butter as culture C2; it appears to be a member of the genus *Aerobacter*. Culture 4, isol-

ated from well-water obtained from a creamery noted for its production of butter with surface taint, and capable of producing this defect under experimental conditions; it appears to be a member of the genus *Pseudomonas*.

Washed suspensions of the cells of these organisms were prepared as previously described (Tarr 1939b) using 0.85 per cent sterile sodium chloride solution, adjusted to approximately pH 7.0 with phosphate buffer, as washing and suspending medium.

ORGANIC BASES

Trimethylamine oxide. Prepared by oxidizing trimethylamine (Eastman Kodak) with hydrogen peroxide, and crystallizing the resulting di-hydrate of trimethylamine oxide $(CH_3)_3N=O.2H_2O$, from ethyl alcohol (Dunstan and Goulding 1899, a and b). The melting point, 96°C., agreed with that of the compound as described. The oxide was further identified by means of its picrate $(CH_3)_3N=O.C_6H_2(NO_2)_3OH$, for which Dunstan and Goulding (1899b) reported as the melting point 196 to 198°C. The present preparation melted at 195 to 196°C.

Triethylamine oxide. Prepared by oxidizing triethylamine (Eastman Kodak, B.P. 88-90°) with hydrogen peroxide (Dunstan and Goulding 1899b). The oxide thus obtained is a very deliquescent crystalline compound, and when exposed to air under normal laboratory conditions exists as a thick syrup containing about 80 per cent of triethylamine oxide as estimated by reducing its aqueous solutions with Devarda's alloy and hydrochloric acid (Lintzel 1934). Whether or not this oxide contains water of crystallization is apparently not known. This compound was identified by means of its picrate $(C_2H_5)_3N=O.C_6H_2(NO_2)_3OH$, with the melting point 164 to 166°C., agreeing with that of Dunstan and Goulding (1899b). (*Note*: On recrystallization the picrate melted at 173°C.).

Tri-n-propylamine oxide. Prepared by oxidizing tri-n-propylamine (Eastman Kodak, B.P. 155-157°) with hydrogen peroxide in ethyl alcohol solution at 60°C. (Dunstan and Goulding 1899b). This compound, like the ethyl oxide, is very deliquescent, and it is apparently not known whether it contains any water of crystallization. Tri-n-propylamine oxide was identified by means of its picrate $(C_3H_7)_3N=O.C_6H_2(NO_2)_3OH$, M. P. 129°C., agreeing with that of Dunstan and Goulding (1899b).

Ergothioneine. Prepared by Prof. Eagles of the University of British Columbia.

Stachydrine. Prepared by Dr. H. B. Vickery, Connecticut Agricultural Experiment Station.

Choline hydrochloride, Betaine hydrochloride and Acetylcholine bromide were Eastman Kodak products.

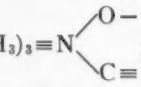
These compounds were dissolved in distilled water, neutralized, where necessary, with sodium hydroxide solution, and sterilized by means of a Seitz filter. The solutions were prepared shortly before use in 0.025, 0.05 or 0.10 M concentration, and were stored at approximately 1.5°C. In the case of the highly deliquescent triethylamine oxide and tri-n-propylamine oxide the strength of the solutions prepared was checked by reducing the oxide by Lintzel's method (1934),

the amount of volatile base formed then being determined. Standard solutions of the other substances used were prepared by weighing the crystalline compounds.

Volatile Base Formation

As in previous experiments (Tarr 1939b) volatile base formation from the various compounds employed was studied only under anaerobic conditions, using Thunberg tubes and relatively aseptic conditions in order to avoid serious external contamination. Each tube received 1 ml. of an 0.025, 0.05 or 0.10 M solution of the organic base; 1 ml. of a solution containing equal parts of 0.1 M glucose and 0.1 M sodium lactate (mixed oxidizable substrate); 1 ml. of 0.2 M phosphate buffer pH 7.0 and 2 ml. of bacterial suspension. The tubes containing the solutions were evacuated thoroughly at a water pump, and were then incubated at 25°C. for about 18 hours. At the end of this time the whole contents of each tube were washed into a laboratory-made "Conway dish" and, after adding 0.5 ml. of strong formaldehyde and 1 ml. of a saturated aqueous solution of potassium carbonate, the amount of volatile base was determined in the usual manner. All experiments were made in duplicate, the results being given in table I.

It will be seen from table I that all six cultures studied exhibited a similar specificity toward each of the organic bases investigated. Trimethylamine oxide and triethylamine oxide were strongly activated and, with the single exception of the action of culture 4 on the ethyl compound, about 80 to 100 per cent of the theoretical amount of volatile base was formed under the experimental conditions. Tri-n-propylamine oxide was also activated, but much less strongly than its methyl and ethyl analogues, only from 2.4 to 17.2 per cent of the oxide being reduced under the experimental conditions. It will be observed that heating the bacteria for 10 minutes at 80°C. destroyed their power of reducing the three oxides. From betaine, choline, acetylcholine and ergothioneine, all of which possess a



grouping, the amount of volatile base formed was, with the single exception of the last-named compound, practically insignificant, and was approximately identical in the case of both unheated and heat-inactivated organisms. It will be seen that a small amount of volatile base (about 7 per cent of the theoretical amount calculated as $(CH_3)_3N$) was recovered from ergothioneine in the case of both unheated and heat-inactivated bacteria. Experiments have shown that this is due to the decomposition of ergothioneine during distillation in alkaline solution. The amount of volatile base formed from stachydrine,

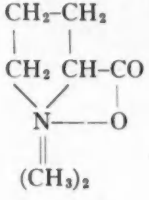


TABLE I. Volatile base formation from different

Nature and concentration of organic base employed	Theor. amount of volatile base N (in γ)	Dry wt. bact. per expt. (mg.)	Unheated bacteria		Heated bacteria	
			Volatile base N recovered (in γ)	Per cent recovery	Volatile base N recovered (in γ)	Per cent recovery
CULTURE 1						
Trimethylamine oxide 0.1 M	1400	16	1411, 1414 → 1413	101	2.6, 3.1 → 2.8	0.20
Triethylamine oxide 0.0815 M	1141	11	1122, 1154 → 1138	98.8	1.4, 0.6 → 1.0	0.08
Tri-n-propylamine oxide 0.051 M	714	18	16.8, 17.4 → 17.1	2.4	1.9, 1.7 → 1.8	0.25
Betaine 0.05 M	700	16	1.7, 1.8 → 1.8	0.25	1.8, 2.4 → 2.1	0.30
Choline 0.05 M	700	16	2.5, 2.2 → 2.4	0.34	2.2, 2.8 → 2.5	0.36
Acetylcholine 0.05 M	700	16	2.8, 3.1 → 3.0	0.42	2.2, 2.8 → 2.5	0.36
Ergothioneine 0.025 M	350	16	24.5, 23.8 → 24.2	6.9	24.9, 24.4 → 24.7	7.1
Stachydrine 0.025 M	350	16	3.1, 2.5 → 2.8	0.80	2.1, 2.1 → 2.1	0.60
CULTURE 18						
Trimethylamine oxide 0.1 M	1400	12	1097, 1123 → 1110	79.2	4.2, 3.6 → 3.9	0.28
Triethylamine oxide 0.0815 M	1141	15	910, 970 → 940	82.3	3.5, 2.8 → 3.2	0.28
Tri-n-propylamine oxide 0.051 M	714	15	21.5, 19.6 → 20.8	2.9	2.2, 1.7 → 2.0	0.28
Betaine 0.05 M	700	12	4.2, 3.6 → 3.9	0.56	4.6, 2.8 → 3.7	0.53
Choline 0.05 M	700	12	4.6, 2.8 → 3.7	0.53	4.9, 5.9 → 5.4	0.72
Acetylcholine 0.05 M	700	12	4.2, 4.2 → 4.2	0.60	4.2, 4.8 → 4.5	0.64
Ergothioneine 0.025 M	350	12	28.3, 26.3 → 27.3	7.8	24.6, 27.2 → 25.9	7.4
Stachydrine 0.025 M	350	12	2.8, 4.9 → 3.9	1.1	3.5, 5.6 → 4.6	1.3
CULTURE 22						
Trimethylamine oxide 0.1 M	1400	17	1392, 1586 → 1389	99.3	4.2, 3.5 → 3.9	0.28
Triethylamine oxide 0.0815 M	1141	11	1170, 1140 → 1155	101	1.4, 1.4 → 1.4	0.12
Tri-n-propylamine oxide 0.051 M	714	10	123, 123 → 123	17.2	1.4, 1.7 → 1.6	0.22
Betaine 0.05 M	700	17	2.0, 2.0 → 2.0	0.29	1.7, 2.5 → 2.1	0.30
Choline 0.05 M	700	17	2.2, 2.8 → 2.5	0.36	2.2, 2.5 → 2.4	0.34
Acetylcholine 0.05 M	700	17	2.1, 2.7 → 2.4	0.34	2.8, 2.2 → 2.5	0.36
Ergothioneine 0.025 M	350	17	21.0, 23.2 → 22.1	6.3	23.5, 22.4 → 23.0	6.6
Stachydrine 0.025 M	350	17	5.6, 4.5 → 5.0	1.4	4.2, 5.0 → 4.6	1.3

substrates by the triamineoxidease of intact bacterial cells.

Nature and concentration of organic base employed	Theor. amount of volatile base N (in γ)	Dry wt. bact. per expt. (mg.)	Unheated bacteria: Volatile base N recovered (in γ)	Unheated bacteria: Per cent recovery	Heated bacteria: Volatile base N recovered (in γ)	Heated bacteria: Per cent recovery
CULTURE C 2						
Trimethylamine oxide 0.1 M	1400	15	1405, 1410 → 1408	101	4.2, 2.2 → 3.2	0.23
Triethylamine oxide 0.0815 M	1411	15	1142, 1135 → 1139	99.8	2.1, 2.5 → 2.3	0.20
Tri-n-propylamine oxide 0.051 M	714	14	122, 123 → 122	17.2	2.2, 2.5 → 2.4	0.34
Betaine 0.05 M	700	15	2.0, 2.2 → 2.1	0.30	2.0, 2.0 → 2.0	0.29
Choline 0.05 M	700	15	1.9, 2.9 → 2.4	0.34	1.8, 2.1 → 2.0	0.29
Acetylcholine 0.05 M	700	15	3.1, 2.8 → 3.0	0.43	4.2, 3.1 → 3.7	0.53
Ergothioneine 0.025 M	350	15	26.3, 25.5 → 25.9	7.4	27.7, 25.7 → 26.7	7.6
Stachydrine 0.025 M	350	15	3.1, 2.8 → 3.0	1.2	3.4, 2.5 → 3.0	1.2
CULTURE C 8						
Trimethylamine oxide 0.1 M	1400	19	1392, 1408 → 1400	100	2.8, 3.5 → 3.2	0.23
Triethylamine oxide 0.0815 M	1411	19	1120, 1150 → 1135	99.4	1.1, 2.5 → 1.8	0.16
Tri-n-propylamine oxide 0.051 M	714	12	118, 120 → 119	16.7	1.7, 2.2 → 2.0	0.28
Betaine 0.05 M	700	19	2.5, 3.1 → 2.8	0.40	2.8, 2.8 → 2.8	0.40
Choline 0.05 M	700	19	3.2, 4.6 → 3.9	0.56	2.9, 3.4 → 3.2	0.46
Acetylcholine 0.05 M	700	19	2.9, 3.2 → 3.1	0.44	2.5, 2.8 → 2.7	0.39
Ergothioneine 0.025 M	350	19	25.8, 26.3 → 26.1	7.5	26.6, 25.9 → 26.3	7.5
Stachydrine 0.025 M	350	19	4.2, 2.8 → 3.5	1.0	2.8, 4.5 → 3.7	1.1
CULTURE 4						
Trimethylamine oxide 0.1 M	1400	9	1124, 1142 → 1133	80.8	3.1, 3.9 → 3.5	0.25
Triethylamine oxide 0.0815 M	1141	7	189, 199 → 194	17.0	2.8, 3.0 → 2.9	0.25
Tri-n-propylamine oxide 0.051 M	714	8	31.1, 29.7 → 30.4	4.3	2.5, 2.5 → 2.5	0.35
Betaine 0.05 M	700	9	2.2, 4.5 → 3.4	0.49	2.8, 2.5 → 2.7	0.39
Choline 0.05 M	700	9	4.5, 4.5 → 4.5	0.64	3.4, 4.4 → 3.9	0.56
Acetylcholine 0.05 M	700	9	3.1, 3.1 → 3.1	0.44	3.5, 3.5 → 3.0	0.43
Ergothioneine 0.025 M	350	9	24.9, 25.2 → 25.1	7.2	25.5, 26.6 → 26.1	7.5
Stachydrine 0.025 M	350	9	4.6, 4.6 → 4.6	0.76	4.9, 3.9 → 4.4	0.80

was also insignificant, and was approximately identical in the case of both unheated and heat-inactivated organisms.

NATURE OF VOLATILE BASE FORMED

Experiments were undertaken in order to ascertain whether activation of the triamine (trialkylamine) oxides being investigated resulted in the formation of the corresponding volatile triamine.

Erlenmeyer flasks (125 ml.) were plugged and sterilized. To each flask was added 20 ml. of washed cells of culture 22 (175 mg. dry wt. of bacteria); 20 ml. of 0.2 M phosphate buffer pH 7.0; 20 ml. of a solution containing equal portions of 0.1 M glucose and 0.1 M sodium lactate (oxidizable substrate), and 20 ml. of an approximately 0.1 M solution of the triamine oxide being studied (*vide infra*). The usual aseptic conditions were observed in these experiments. The reaction mixtures were incubated at 25°C. for about 24 hours under anaerobic conditions, using partial vacuum and alkaline pyrogallol to absorb the oxygen. The contents of each flask were examined separately as follows.

Trimethylamine oxide (20 ml. of an 0.1 M solution used). The solution was washed into a small Claissen flask, 2 ml. of strong formaldehyde and 5 ml. of saturated potassium carbonate solution were added, and approximately 10 ml. of distillate were collected in 10 ml. of ice-cold 6 per cent hydrogen peroxide by distilling *in vacuo* at about 60°C. The resulting solution was permitted to stand for one day at room temperature, and was then evaporated to a thick syrup, first over sulphuric acid and finally over phosphorus pentoxide. The syrup (150 mg.) was dissolved in 1 ml. of absolute ethyl alcohol, 10 ml. of dry, re-distilled ether were added and the supernatant liquid was decanted from the resulting precipitate. To the white gummy residue (127 mg.) 2.5 ml. of a saturated aqueous solution of picric acid were added. A crystalline picrate separated in yellow needles. After cooling to about 0°C. the precipitate was collected on a small filter and was dried for a short time at 110°C., 34 mg. of crystals being obtained, M.P. 194-195°C. On mixing with crystals of trimethylamine oxide picrate (M.P. 195-196°C.) a mixed M.P. of 195° was obtained. The yield of picrate was very low, being only 5.6 per cent of that expected by theory. Probably factors such as the loss of trimethylamine during distillation *in vacuo*, the incompleteness of oxidation of the very dilute solution of the base in the hydrogen peroxide solution and the solubility of the picrate in water were in part responsible for the low recovery.

Triethylamine oxide (20 ml. of 0.091 M. solution used). The procedure adopted for recovering the base was practically identical with that employed in recovering the methyl analogue. In this case the picrate was known to be less soluble in water, so the distillate was merely concentrated to approximately 10 ml., 20 ml. of a saturated aqueous solution of picric acid were added, and the solution cooled to about 0°C. The crystalline picrate which formed was dried and weighed as in the case of the methyl compound, a yield of 188 mg. being obtained. The filtrate from the first crop of crystals was concentrated to about 12 ml. over sulphuric acid, and more crystals (64 mg.) were isolated. Both fractions melted at 161 to 162°C. When mixed with triethylamine oxide picrate,

M.P. 164 to 166°, a mixed melting point of 163° resulted. The total yield of picrate thus obtained (252 mg.) was 41 per cent of that expected, presuming complete reduction of triethylamine oxide in the solution and no loss in recovery.

Tri-n-propylamine oxide. Several attempts were made to recover tri-n-propylamine by methods similar to those followed in the case of the methyl and ethyl oxides, but without success. The reason for this failure is undoubtedly because tri-n-propylamine oxide is but feebly reduced in comparison with its methyl and ethyl analogues. This fact is shown well in table III. By using very much larger proportions of reagents it might be possible to obtain sufficient tri-n-propylamine for purposes of identification. It is important to note in this connection that the characteristic odour of tri-n-propylamine is evident when reaction mixtures such as those employed in these experiments are made alkaline. There would seem little doubt, therefore, that tri-n-propylamine is the product of bacterial reduction of the corresponding oxide.

UNITY OF ACTIVATING ENZYME

Experiment 1. The following solutions were added to each of twelve Thunberg tubes: 1 ml. of a suspension of culture 22 (9 mg. dry wt. of bacteria), 1 ml. of 0.2 M phosphate buffer pH 7.0, 1 ml. of a solution containing equal portions of 0.1 M glucose and 0.1 M sodium lactate, and 2 ml. of triamine oxide solution. The amount and kind of triamine oxide was varied, three tubes being prepared in each case (see table II).

TABLE II. Volatile base formation from trimethylamine oxide, triethylamine oxide, and from a mixture of these compounds.

Triamine oxide employed and its concentration (5 ml. of solution)	Theoretical amount (in γ) of volatile nitrogen for complete reduction of the oxide	Volatile nitrogen recovered (in γ) after:		
		3½ hr.	4½ hr.	6 hr.
Methyl, 0.02M	1400	685	926	1115
Methyl, 0.04M	2800	660	915	1130
Ethyl, 0.02M	1400	701	940	1150
Methyl, 0.02M plus Ethyl, 0.02M	2800	693	910	1138

The tubes were evacuated and placed in a 25°C. thermostat, the amount of volatile triamine nitrogen being determined at intervals on the whole contents of one tube of each of the four varieties. From the results given in table II it will be seen that in this experiment the enzyme was saturated with respect to its substrate in 0.02 M trimethylamine oxide solution, there being no increase in volatile base formation with 0.04 M trimethylamine oxide; also the amount of volatile base nitrogen formed from triethylamine oxide was about the same as that formed from the trimethylamine oxide. If different enzymes were concerned in the activation of these two oxides, the amount of volatile base formed in the case of the solution containing both trimethylamine oxide and triethy-

lamine oxide would be expected to be approximately double that formed in a solution of corresponding concentration of either one of these compounds separately. In this experiment the amount of volatile base is about the same in the mixed solution of oxides as it is in the case of a single oxide, and it must therefore be assumed that the same enzyme is responsible for the activation of both trimethylamine oxide and triethylamine oxide.

Experiment 2. The technique adopted was identical with that followed in the foregoing experiment, using 0.02 M trimethylamine oxide, 0.02 M tri-n-propylamine oxide, and a suspension of culture 22 containing 7 mg. dry weight of bacterial cells per experiment. The results, recorded in table III, show that the propyl oxide is activated to a very much smaller extent than is the methyl oxide, only 2.4 per cent as much volatile nitrogen being recovered over a five-hour period. Also, the amount of volatile base nitrogen formed in the case of the mixed solution of trimethylamine oxide and tri-n-propylamine oxide was actually less than that formed from the methyl oxide alone. This may be due to a toxic effect exerted by the propyl radicals, to the fact that the enzyme was saturated with an excess of propyl oxide, to the fact that the adsorption of the propyl oxide by the enzyme hindered the activation of the methyl oxide, or to some similar cause. Until further work is done in order to determine the affinity of this enzyme for the substrates in question it will be imposssible to state definitely whether the propyl oxide is activated by the same enzyme as the methyl oxide.

TABLE III. Volatile base formation from trimethylamine oxide, tri-n-propylamine oxide, and from a mixture of these compounds.

Triamine oxide employed and its concentration (5 ml. of solution)	Theoretical amount (in γ) of volatile nitrogen for complete reduction of the oxide	Volatile nitrogen recovered (in γ) after 5 hrs.
Methyl, 0.02M	1400	512, 496 — 504
N-propyl, 0.02M	1400	12.3, 11.9 — 12.1
Methyl, 0.02M plus N-propyl, 0.02M	2800	471, 451 — 461

DISCUSSION

The experiments herein described show that the enzyme which activates trimethylamine oxide exhibits a well-defined substrate specificity. Thus it will activate at least the lowest three members of the homologous series of compounds having the general structure $R_3N=O$, which might be regarded as o-alkyl dialkyl hydroxylamines, $R-N\begin{smallmatrix}O-R\\R\end{smallmatrix}$. On the other hand compounds which possess the $(CH_3)_3N\begin{smallmatrix}O-\\C\equiv\end{smallmatrix}$ grouping are not activated. As yet no attempt has been made to ascertain whether other alkyl-substituted hydroxylamines, such as αβ-diethyl, ββ-diethyl and ββ-dipropylhydroxylamines are activated with the forma-

tion of volatile base. Shewan (1938) has found small amounts of dimethylamine in spoiling fish muscle, but since attempts to prepare a $\beta\beta$-dimethylhydroxylamine failed (Dunstan and Goulding 1899b) it seems unlikely that this compound is the precursor of dimethylamine.

Experiments have strongly indicated that the enzyme which activates trimethylamine oxide is the same as that which activates triethylamine oxide. So far attempts to prove that the same enzyme activates tri-n-propylamine oxide have failed, but the fact that the six different cultures all activated the three oxides to about the same degree makes it highly probable that one enzyme is involved. In view of these findings, and the specificity of the enzyme toward triamine oxides, the name "Triamineoxidease" appears to be the most fitting designation for the activating principle.

The results described in this paper are of interest in that Beatty (1938), basing his findings on the fact that Lintzel's chemical method of reduction (1934) does not cause volatile base formation from choline, betaine, γ-butyrobetaine and carnitin, stated that at least 94 per cent of the trimethylamine in spoiling fish muscle arises from trimethylamine oxide. He also showed qualitatively that trimethylamine is formed during the bacterial spoilage of fish muscle press juice.

It must be emphasized in closing that all the experiments described have been carried out under anaerobic conditions. It is not unlikely that the breakdown of certain of the bases employed would follow a different course under aerobic conditions.

SUMMARY

The bacterial enzyme previously described (Tarr 1939, a and b) which activates trimethylamine oxide to form the reduction product, trimethylamine, also activates triethylamine oxide and tri-n-propylamine oxide with formation of the corresponding volatile bases. It does not activate the $(CH_3)_3N\langle^{O-}_{C\equiv}$ group of betaine, choline, acetylcholine or ergothioneine; nor the $(CH_3)_2N\langle^{O-}_{CH}\langle_{CH_2-}$ group of stachydrine.

In view of these results, and the specificity of this enzyme toward triamine oxides, it is suggested that it be named Triamineoxidease.

Triamineoxidease exists in the cells of bacteria from widely different sources, including spoiling fish muscle, well water and surface taint butter. It has been demonstrated in organisms from five different genera.

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